BRINE ORGANISMS AND THE QUESTION OF HABITAT-SPECIFIC ADAPTATION

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ABSTRACT

Among the well-known ultrasaline terrestrial habitats, the Dead Sea in the Jordan Rift Valley and Don Juan Pond in the Upper Wright Valley represent two of the most extreme. The former is a saturated sodium chloride-magnesium sulfate brine in a hot

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desert, the latter a saturated calcium chloride brine in an Antarctic desert. Both Dead Sea and Don Juan water bodies themselves are limited in microflora, but the saline Don Juan algal mat and muds contain abundant nutrients and a rich and varied microbiota, including Oscillatoria, Gleocapsa, Chlorella, diatoms, Penicillium and bacteria.

In such environments, the existence of an array of specific adaptations is a common, and highly reasonable, presumption, at least with respect to habitat-obligate forms. Nevertheless, many years of ongoing study in our laboratory have demonstrated that lichens (e.g. Cladonia), algae (e.g. Nostoc) and fungi (e.g. Penicillium, Aspergillus) from the humid tropics can sustain metabolism down to -40°C and growth down to -10°C in simulated Dead Sea or Don Juan (or similar) media without benefit of selection or gradual acclimation. Non-selection is suggested in fungi by higher growth rates from vegetative inocula than spores. The importance of nutrient parameters was also evident in responses to potassium and reduced nitrogen compounds.

In view of the saline performance of tropical Nostoc, and its presence in the Antarctic dry valley soils, its complete absence in our Don Juan mat samples was and remains a puzzle.

We suggest that adaptive capability is already resident in many terrestrial life forms not currently in extreme habitats, a possible reflection of evolutionary selection for wide spectrum environmental adaptability.

INTRODUCTION

Although long a part of conventional ecology and biogeography (see, for example, 1), extreme environments biology acquired new and special significance with the advent of exobiological interests (2-7). The focus of this interest on Mars (8) in turn called attention to cryobiology, especially of the Antarctic dry valleys (9, 10). From its discovery (11), Don Juan Pond in the Wright Valley has offered a model for habitats combining extreme salinity with low temperature, but its rich and complex mat biology has only recently been described fully (12, 13).

In general, the presence of organisms in these cold brine environments is assumed to be the result of extended processes of specific selection and adaptation among populations native to more general, hence less specific habitats.

Certainly, the conventional view holds that life arose under warm, non-saline conditions, whatever else the chemical requirements may have been.

Experiments in this laboratory spanning nearly 20 years (4, 5, 16, 14, 15) have revealed extraordinary performance capabilities among common organisms placed in extreme environments. Included are more recent findings which prompt us to question the assumption that the extended processes of selection associated with successful adaptation of organisms to extreme environments are in fact habitat-specific and habitat limiting.

MATERIALS AND METHODS

Organisms.--In the experiments described and discussed here, four species have been used.

Cladonia skottsbergii was collected in Hawaii on moist lava cinder substrata at surface temperatures of 28-42°C. Air temperatures of 12-26°C and relative humidities of 75-100% were common.

Penicillium notatum was subcultured from decaying fruit and maintained at 24-25°C on Czapeks broth.

Nostoc sp. was collected in mixed cultures in gelatinous masses with green algae, diatoms and bacteria. Although impure, the chlorophyll a:b ratio is about 50, showing the predominance of blue-green organisms. They were located on a moist cement floor in an unused greenhouse. The air temperature was 26-34°C and the humidity 80-100%.

Dunaliella salina, a motile green alga, was obtained from lagoon waters near Honolulu at a temperature of 22-28°C and 3200-4200 ppm salinity.

Our experimental materials thus, include a heterotroph and three photoautotrophs, one symbiotic, and both prokaryotic and eukaryotic forms.

Experimental Procedures -- General cryobiological culture methods have been detailed for fungi (14, 15); for algae (16-20); and for special liquid ammonia experiments (21).

Isotope uptake experiments have been described for ^{14}C-Carbonate (19, 20); ^{14}C-labelled acetate, glucose and leucine (18); and tritiated uridine, thymidine and amino acids (21).

Carbon dioxide production in Cladonia and Penicillium was measured by infra-red gas analysis.

Photoautotrophs were maintained under cool white flourescent illumination at 150-200 Einsteins·cm^{-2}·sec .

Data are based on replicated measurements (at least 3), and are given as mean value standard deviation.

RESULTS

An ordinary moist Cladonia thallus at 20°C produces CO_2 at about 600 mm^{3}g^{-1}h^{-1}· (6). Lichens are not commonly found in nature under water, but submerged Cladonia respires at a high rate (Table 1). On salt solutions, respiration falls with increasing ion coordination and decreasing water activity except in calcium chloride which was less inhibiting than expected. The frozen thallus in salt-free media, sodium or potassium salts at -40° C was inactive. In the unfrozen brines, respiration continued, and was especially high in $CaCl_2$, about 10% of salt-free 20°C control.

Table 1
Respiration of the Lichen Cladonia in Salt Solutions

Salt	Water Activity (Saturated Solution, 20°C)	CO_2 Production* mm^3·g^{-1}·h^{-1} 20°C	-40°C
None	1.00	460±62	0
KCl	0.85	387±44	0
NaCl	0.76	300±44	0
$MgCl_2 \cdot 6H_2O$	0.33	138±19	10±4
$CaCl_2 \cdot 6H_2O$	0.29	266±31	48±8
$LiCl \cdot H_2O$	0.12	145±28	27±6

*Carbon dioxide production was measured after 4 weeks in water or saline media.

At -6°C, Penicillium does not grow in ordinary hard frozen, low salt media.

At 6°C, biomass production approached 2g·l^{-1}·d^{-1} and CO_2 about 80 mm^3·g^{-1}·h^{-1} (Table 2); cultures sporulated readily and were not stimulated by K-ion.

At -6°C in the Dead Sea and Don Juan Pond media macroscopic colonies were present only after 18 months incubation. Growth

was enhanced somewhat by K-ion supplement, but little affected by replacement of nitrate by amino-N.

Table 2
Growth and Respiration of Penicillium in Brine Salt Media at -6°C

Brine Salts	Nitrogen Source	KCl (M)	Growth* Spores mg l d		CO_2 $mm^3 \cdot g^{-1} \cdot h^{-1}$
6°C, low Salt Control	NO_3^-	0.035	1860±220	+	81±11
	NO_3	0.35	1905±207	+	86±12
Dead Sea ($NaCl-MgSO_4$)	NO_3^-	0.035	81± 11	-	16± 2
	NO_3	0.35	104± 31	+	17± 2
	Amino	0.35	117± 46	+	22± 3
Don Juan ($CaCl_2$)	NO_3^-	0.035	99± 30	-	23± 5
	NO_3	0.35	128± 38	-	26± 5
	Amino	0.35	132± 41	+	28± 6

*Cultures were inoculate from a single young vegetative mycelium stock. Non-saline controls were harvested after 3 weeks; saline cultures were harvested after 30 months. All cultures received 1% glucose.

Nostoc cultured at -20°C in Don Juan Pond media fixes ^{14}C-acetate and ^{14}C-leucine above and beyond the non-specific labelling seen in killed cells (Table 3). Correcting for label in killed samples, light has negative effects on fixation or none initially, but increases C-levels from both sources after 8 days.

Mixed Nostoc cultures (Figure 1) incorporate ^{14}C-glucose readily under Don Juan Pond simulation, -20°C and 34% Ca-salt brine (Figure 2). The label appears mainly in wall and other extracellular products, but may also appear within cells (Figure 3).

Pure cultures of Dunaliella remain motile down to about -15°C in 24% NaCl (22) and retain the ability to fix $^{14}CO_2$ at -8 C (Table 4).

In addition to the foregoing studies which concern uncommon, but by no means rare, terrestrial low temperature, low water availability environments, we call attention to a truly exotic culture medium in which there is no unassociated liquid water and extreme cold (21). Conidiospores of Penicillium were incubated

in liquid ammonia stabilized with glycerol, the whole containing about 1% water and a complex nutrient (Table 5). Tritium labelled precursors of nucleic acid and protein were included. Cultures were "incubated" at -40°C for 6 months. Controls included both heat and -radiation killed spores to correct for non-specific uptake.

Relative to the lethal ionizing radiation, some uptake occurred in heat treated spores, but 5-fold more trichloracetic acid-insoluble label was found in "normal" inocula. Visual (optical) evidence for the living condition, germ tube formation, occurred with a frequency of 1-10 per thousand and was not observed at all in heat- or radiation-treated inocula.

Somewhat parallel observations of labelled thymidine incorporation into DNA and of leucine into protein have been reported for common Oscillatoria species after 40 days at 25°C in a Don Juan Pond brine (23). Leucine incorporation was enhanced in light.

Among the biota in Don Juan mat samples, Oscillatoria, Chlorella, and Penicillium (12, 13) were readily cultured in ordinary media at 20-25°C. Recently, the fungus Cladosporium was added to this list. An intensely orange Gleocapsa found has not been cultured in warm, low-salt media; diatoms have not grown under any conditions.

DISCUSSION

Nearly 20 years ago, an exobiological discussion of exotic biochemistry concluded "careful reflection about each of the properties of water reveals that no matter how they are woven into the Earth-pattern of life, none of them seems to involve a functional uniqueness that would preclude life without that property" (8, p. 246).

If this was a sound speculation then, it must be viewed as well on the way to substantiation now.

At the biochemical level, enzyme activities have been demonstrated in methanol or saturated aqueous LiCl at temperatures of -18 to -48°C; in nitromethane; in saturated aqueous ammonia, and in 1 normal sodium hydroxide (10, 24, 25, 26).

Organized metabolism--photosynthesis, respiration, substrate incorporation, macromolecular biosynthesis--have been documented here and elsewhere (as cited) under conditions which limit severely the availability of water.

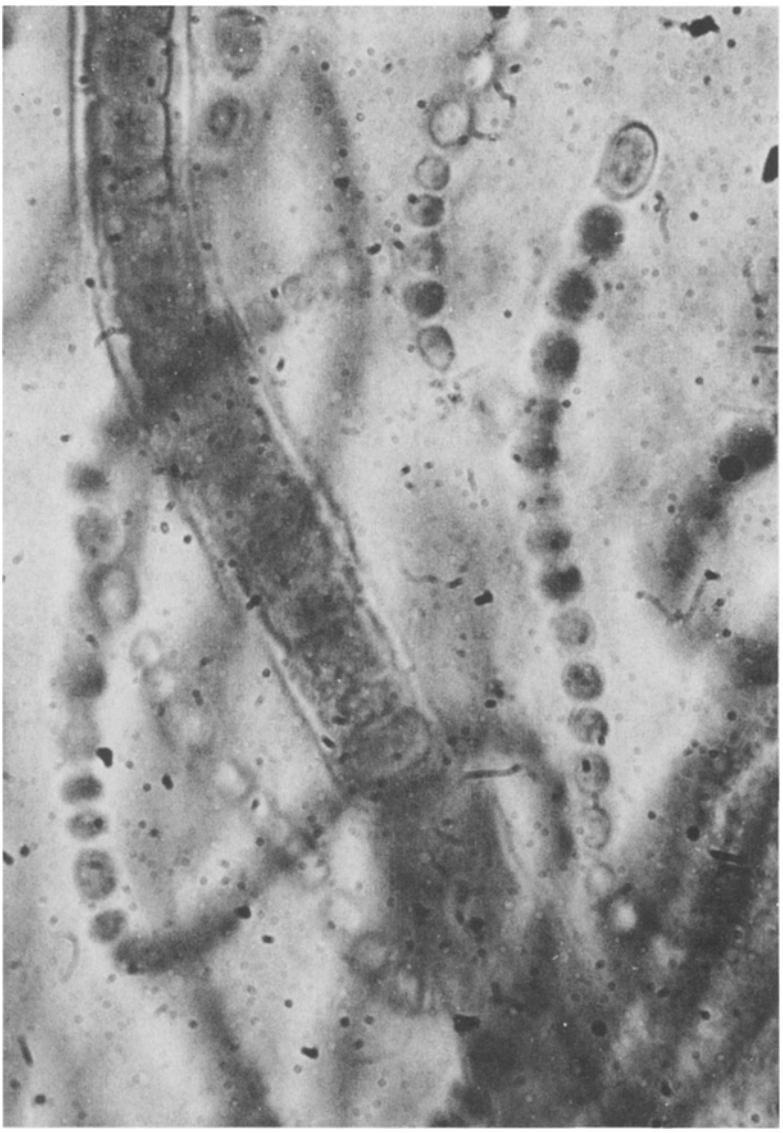

Figure 1. Mixed culture showing filaments of Nostoc and green algae. Original magnification 950x.

Table 3
Incorporation of ^{14}C-labelled Organics into Mixed Tropical Algal Cultures Rich in Nostoc After Preculture for 4 weeks at -20°C

Time (days)	Light $E \cdot cm^{-2} \cdot sec^{-1}$	^{14}C Activity (in 10^3 cpm/ml)* ^{14}C-Acetate		^{14}C-Leucine	
		Killed**	Living	Killed**	Living
1	0	1.1±0.2	9.5±1.0	0.8±0.2	4.7±0.6
	165±3	0.9±0.1	6.0±0.7	0.9±0.2	4.5±0.6
8	0	1.0±0.2	6.4±0.8	0.9±0.2	5.0±0.6
	165±3	1.3±0.3	8.1±0.9	1.2±0.2	7.7±1.3

*^{14}C-labelled compounds were supplied at 0.1 Ci/ml m media. Biomass collected on millipore filters was assayed using a Packard Scintillation Counter.
**Killed by autoclaving.

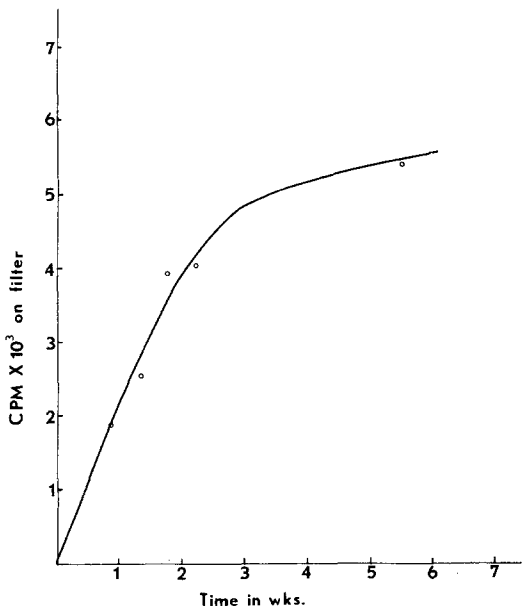

Figure 2. The time course of ^{14}C-glucose uptake by mixed cultures of Nostoc in simulated Don Juan Pond media at -20°C, 34% salinity, for labelling details, see footnote, Table 3.

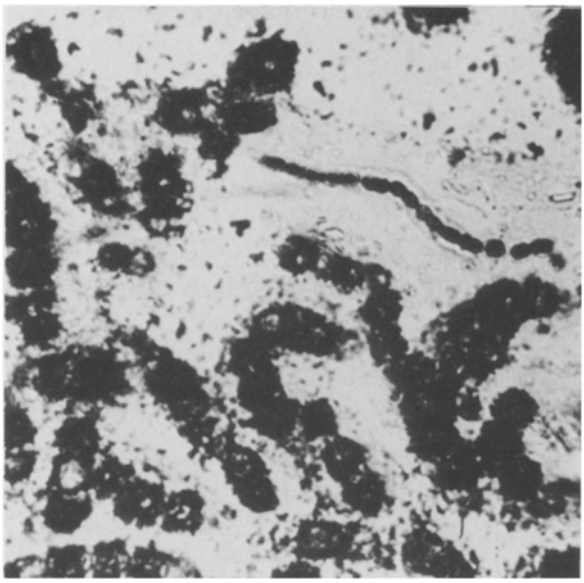

Figure 3. Unstained autoradiograph of algal filaments after 2 weeks in ^{14}C-glucose (0.1 Ci/ml) Don Juan Pond-water.

Table 4
Incorporation of Carbonate ^{14}C into Dunaliella as a Function of Temperature in 15% NaCl

Temperature °C	^{14}C-Activity in Cells+ after 30 h (10^6 cpm/ml)	
-8	0.09 ± 0.02	(3.2%)
-1	0.87 ± 0.23	(31%)
6	1.9 ± 0.3	(68%)
25	2.8 ± 0.4	(100%)

*Supplemented with Hutner's marine Chlamydomonas medium for labelling details see footnoe, table 3. Cultures were incubated under cool white fluorescent light at 150 Einsteins $\cdot$ cm$^{-2}\cdot$sec^{-1}.

Table 5
Incorporation by Penicillium Conidia of TCA-Insoluble Tritium Labelled Metabolites in Glycerol-NH_3 Media After 6 months at 40°C

Tritium Label as	Treatment+ None	125° for 24h	5Mrad ^{60}Co
		(cpm/mg)	
^{3}H-Thymidine	143±10	168±8	10±2
5,6-^{3}H-Uridine	162±12	48±6	19±3
2,3-^{3}H-L-Phenylalanine and ^{3}H-L-Leucine	134±10	23±3	28±3

*The medium was 60% liquid NH by volume with only 1.2% water and a glucose-peptone-yeast extract nutrient for isotopic study details, see ref. 21.

Finally, the existence of natural terrestrial extreme environments as habitats, even habitats rich and varied in biological diversity, challenges the traditional views of water as a vehicle and medium for life.

We do not challenge the essentiality of the liquid state for life, nor the importance of structural water in biomolecules. In doubt is the perception that the abundance of bulk water is in itself essential for life.

This distinction is made obvious when it is considered that one of the biotically richest extreme environments, the Don Juan Pond mat, is perhaps the "brinyest" and coldest liquid habitat known.

When an aqueous environment allows only 10 moles of water for each mole of $CaCl_2$ solute with its high coordination, and at temperatures well below 0°C, H_2O per se seems almost to be a minor nutrient, whereas the highly associated, coordinated liquid phase remains.

We obviously do not know what the boundary conditions are for contemporary terrestrial life, much less protolife, save that they are remote in most respects from commonly perceived norms. Our prescription would include, of course some small amounts of water and a temperature compatible with the liquid state.

If the conditions for the existence of life can be so remote from our general perceptions, then what of habitat-specific adaptation to such extremes?

As we have shown, not only can an assortment of Don Juan Pond organisms be cultured directly under fully conventional conditions, but organisms with a recent history of benign habitats can in turn perform remarkably well under the rigors of experimental extremes including Don Juan simulation.

Clearly some--indeed most--terrestrial organisms are incapable of such "acrobatics". But the fact that some can do so also challenges the concept that selection for life in extreme or specialized habitats demands concomitant reductions in adaptive potential.

This view is an extension to extreme habitats of that recently set forth for island ecosystems (27), that localized populations of island species can be fully as genetically polymorphic as those of widespread continental species. That such specialized populations remain highly competent for adaptive evolution suggests that the heritable quality is adaptivity itself, not a set of specific adaptive characters.

The astronomical variety of environmental combinations that have existed on Earth since the earliest stages of biopoesis obviously have acted to confer upon some species the ability to cope with habitats far removed from their c-nrent associations, and perhaps outside of their specific evolutionary experience.

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