Salinity and the Ecology of *Dunaliella* from Great Salt Lake

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SUMMARY

Studies are reported on the distribution and salinity tolerances of populations of *Dunaliella*, a eucaryotic alga, in Great Salt Lake, Utah, U.S.A. This lake provides salinities varying from about 10 % (w/v) NaCl to saturated (greater than 30 % w/v). The alga is found throughout this salinity range, although population density varies markedly, mainly because of the influence of grazing animals in waters of low salinity. Enrichment cultures were set up using a range of salinities; at the lower salinities a wide variety of algae grew, but at the higher ones only *Dunaliella* was obtained. However, cultures derived from saturated brine and grown at salinities of around 25 % (w/v) were not optimally adapted to these conditions, but grew and photosynthesized better in 10 to 15 % (w/v) NaCl. A natural population from a saturated brine also had an optimum at a lower salinity than its habitat. It is concluded that although this eucaryotic alga is able to grow in saturated brine better than any other alga, it is not optimally adapted to these conditions and is apparently able to maintain populations at high salinity only because it meets no competition from other algae.

INTRODUCTION

Members of the eucaryotic algal genus *Dunaliella* are widespread in saline environments (Teodoresco, 1905, 1906; Hof & Freymy, 1933; Lerche, 1937; Volcani, 1944; Smith, 1950; Butcher, 1959), and have been much studied, perhaps because of the striking pink to red coloration which they sometimes impart to waters and sediments. The genus *Dunaliella* was first separated from *Chlamydomonas* by Teodoresco (1905) on morphological grounds, and since then a number of species have been described (Butcher, 1959). These occur in diverse habitats, from almost fresh water to saturated brine, although the salinity requirements of different species have not been specified. Most attention has been paid to cultures able to grow in media of greater salinity than sea water. The mechanism by which *Dunaliella* withstands high salt concentrations differs from that of the halobacteria (Kushner, 1968; Larsen, 1967). In the latter, it appears that the salt concentration (primarily potassium, with some sodium) inside the cell is higher than the external one, permitting water to flow in, and the enzymes of the bacteria are unusually resistant to high concentrations of salt. In *Dunaliella*, high concentrations of glycerol are synthesized intracellularly through photosynthetic processes (Wegmann, 1971) and the alga maintains an intracellular glycerol concentration such that the intracellular osmotic pressure is higher than the extracellular pressure (Ben-Amotz & Avron, 1973), thus permitting the alga to take up water even when growing at high salt concentrations. The intracellular salt concentration is lower than the external concentration, and intracellular enzymes of *Dunaliella* are actually inhibited by high salt concentrations (Johnson *et al.* 1968).

Despite the evolutionary and ecological interest in this alga, little work has been done in relating its salinity requirements to those of the habitats in which it lives. Gibor (1956)

cultured *Dunaliella* from solar-salt ponds and determined the optimum salinity requirements. A strain from saturated brine, although able to grow at this salinity, had an optimum at about 3 to 4 times the salinity of sea water (10 to 15 %, w/v, NaCl). Other strains isolated from salt ponds of lower salinity also showed optimal growth at 3 to 4 times sea salinity but were unable initially to grow in saturated brine, although they could be adapted to this high salinity by successive upward transfers. All of Gibor's isolates of *Dunaliella* grew quite well at sea-water salinity. Kirkpatrick (1934) set up enrichment cultures of Great Salt Lake material in media of various salinities and showed that *Dunaliella* (called by her *Chlamydomonas*) was able to grow over a wide range of salinities up to saturation. However, she did not determine the optimum salinity for the cultures isolated. Recently, Van Auken & McNulty (1973) determined the optimum salinity for *Dunaliella* isolated from Great Salt Lake but did not specify the salinity of the water from which the isolate was obtained, although the culture was isolated on a medium containing 21 % (w/v) NaCl and proved to have a sodium chloride optimum of 19 % (w/v). Hof & Frey (1933) studied the halophilic blue-green algae, but noted that *Dunaliella* was of common occurrence and even more resistant to storage in dry salt than were the blue-greens. They were able to culture *Dunaliella* from dry solar salt that had been stored for up to seven years.

In the present work, as a means of generalizing the salinity responses of the organism, the concept of water potential is used, which expresses the water availability. Water potential can be expressed in a variety of pressure units, although the unit most commonly used is the bar, which is approximately equivalent to an atmosphere, or to 100 joules/kg (Griffin, 1972); 1 bar = 10^5 Pa. A water potential of -100 bars is equivalent to about 12 % (w/v) NaCl, and -200 bars to about 19 % (w/v) NaCl.

Although considerable work has been done on both matric and osmotic water potential effects on bacteria and fungi (Scott, 1957; Griffin, 1972; Corry, 1973), much less has been reported on algae, despite the fact that in many habitats (e.g. subaerial, desert, marine, hypersaline, soil) water potential may be a major factor controlling algal growth and activity. By using the water-potential concept it is possible to compare the ability of algae from such diverse kinds of environments of low water potential as deserts and salt lakes to tolerate reduced water potential. Smith & Brock (1973) studied the effect of water potential on the thermophilic alga *Cyanidium caldarium* in geothermal soils, and developed methods applicable to the study of algae in other systems. This work was extended to a study of the blue-green alga *Microcoleus vaginatus* from a desert crust (Brock, 1975a), and the coccoid green alga, *Trebouxia* sp., present in aerial and terrestrial lichens (Brock, 1975b). In all of these studies, the algae were shown to be quite sensitive to lowered water potential, being unable to develop at water potentials below -20 to -100 bars.

The present paper relates the salinity requirements of *Dunaliella* to the salinities of the natural environments in which it lives.

METHODS

Cultures. Most of the work was done with strain 123-4, isolated in August 1970 from salt collected at Bridger Beach off Antelope Island, Great Salt Lake, Utah, U.S.A. In the sand a depression had formed which contained crystalline salt, dry on top but saturated with brine underneath, and the salt was visibly green from large accumulations of actively motile cells of *Dunaliella*. In August 1970 the density of the lake water corresponded to a salt concentration equivalent to about 20 % (w/v) NaCl. The brine from which the alga came, being saturated, had over 25 % (w/v) NaCl. Boiled Great Salt Lake water was used as the basis of

Table 1. Sodium chloride concentrations used to achieve various water potentials

NaCl (molality)	Weight (g/100 ml)	Percentage equivalent (w/v)	Water potential (bars)
0.15	0.87	0.87	-7
0.61	3.54	3.5	-28
1.2	6.96	6.8	-56
2.8	16.2	15.0	-145
4.0	23.2	19.3	-210
5.2	30.1	24.3	-307

Water potentials were calculated from the formula of Lang (1967).

a culture medium, to which 0.1 vol of tenfold concentrated mineral salts medium D of Castenholz (1969) was added. Culture tubes 18 mm in diameter with stainless steel caps were used, and incubation took place near a north-facing window. Within three or four days the culture was visibly green, and consisted exclusively of *Dunaliella* cells. The culture was maintained, with transfer at about three-month intervals, from August 1970 until August 1973, when the present experiments were initiated. The culture remained viable even after the water of the medium had evaporated and crystalline salt formed.

In June 1974 a more extensive series of enrichment cultures were set up, using material collected from various locations on the lake. The mineral salts medium of Allen (1959), adjusted to pH 7.5 to 8.0 and supplemented with NaCl to the desired concentrations, was used. Incubation was at 25 to 30 °C, with illumination from fluorescent lights (about 200 ft candle). No attempt was made to obtain axenic cultures, although in the autotrophic medium used bacterial population densities were generally low.

For obtaining larger volumes of cultures, 1 l flasks with aluminium foil or stainless steel caps, containing 250 ml medium were incubated as above. The cultures were periodically swirled or shaken.

Cell counts were performed using an American-Optical haemocytometer.

Photosynthesis was measured using a ^{14}C technique. Cell suspensions (4 ml) were placed in 5 ml screw-capped vials. In most experiments, $\text{NaH}^{14}\text{CO}_3$ (20 $\mu\text{Ci/ml}$; 10 $\mu\text{g}/\mu\text{Ci}$) was used at a concentration of 2 $\mu\text{Ci/vial}$. At the end of the experiment, 0.5 ml of 40 % (w/v) formaldehyde was added. The contents of each vial were then passed through a membrane filter (Millipore, 0.45 μm), the filter washed with medium containing 20 % (w/v) NaCl, dried, and counted either in gas-flow or liquid-scintillation counter systems.

Salinity measurements of natural habitats. In the field, salinities were determined approximately with a wide-range hydrometer (Fisher Scientific, Chicago, Illinois, U.S.A.) which read up to a density of 1.250 g/ml. Water samples were then collected and more precise determinations made in the laboratory by weighing known volumes of water in 10 ml volumetric flasks and calculating salinity using tables from the *Handbook of Chemistry and Physics* (1974), assuming that the variant solute was NaCl. (Great Salt Lake water does contain higher than normal concentrations of magnesium, calcium and potassium, but to a first approximation the controlling solute can be considered to be NaCl.)

To determine total dissolved solids, water samples were dried overnight at 105 °C, and the dry weight of salts formed determined.

Water potential calculations. Water potential was calculated using the equations and table given by Lang (1967). The relationship between water potential and NaCl concentration is given in Table 1.

Table 2. *Salinity characteristics of various Great Salt Lake habitats*

Location	Date	Density (g/ml)	Total dissolved solids (%, w/v)
Northern arm			
Spring Bay	12 June 1974	1·239	29·6
Spring Bay	17 July 1974	1·2065	34·94
Rozell Point	12 June 1974	1·2175	32·75
Southern arm			
East side, near Syracuse, Utah	13 June 1974	1·0735	12·3
North end of Antelope Island	30 August 1970	1·148	20·0
	13 June 1974	1·063	18·1
South shore, Silver Sands Marina	18 July 1974	1·0719	12·2

RESULTS

The habitat

Great Salt Lake, which has an estimated age of 6500 years (Langbein, 1961) is a remnant of the much larger Lake Bonneville, which during the Pleistocene occupied the present basin and overflowed at Red Rock Pass into the Portneuf, Snake and Columbia Rivers (Hahl, Wilson & Langford, 1965). It is the largest lake in the Western Hemisphere with no outlet to the sea. Fresh water enters the lake primarily from rivers draining the high mountain ranges to the east, since the western and northern borders are surrounded mainly by low mountains where the rainfall is low. The chemical characteristics of the lake water change with water level. Although there has been an overall downward trend in the level of the lake since the early 1870s, short-term fluctuations in water level occur, and currently (1974) the lake is at one of the highest levels for many years (W. Katzenberger, Utah Geological and Mineralogical Survey; personal communication). Nowadays, Great Salt Lake essentially consists of two separate bodies of water, for in 1957 to 1959 a rock-fill causeway was constructed across the middle of the lake which prevents the less salty water from the southern portion of the lake from moving as before into the northern portion. Because of lake level and density differences and the restricted movement of water from the southern section (where the rivers enter) to the northern, there has been a progressive increase in the concentration of salt in the northern portion of the lake and a decrease in the southern portion (Madison, 1970; Whelan & Stauffer, 1972). In addition, on the eastern and southeastern side of the lake (where the major rivers actually enter) there is a further opportunity for dilution of lake water. Thus a series of salinities exists in the lake basin, from virtually fresh water to saturated brine where NaCl is precipitating out. Measurements at a series of locations in the summers of 1970 and 1974 are shown in Table 2. The marked difference between the southern and northern arms is readily apparent. In the northern arm, NaCl was precipitating out and the total dissolved solids content was over 30 % (w/v), whereas in the southern arm salt precipitation did not occur and the concentrations ranged from 12 to 20 % (w/v). The dilution of southern-arm water over the past five years is shown by analyses made from water collected off the north end of Antelope Island (away from the point where river water enters the lake); the total dissolved solids has dropped from 20 to 18 % (w/v) during this time.

Dunaliella is present throughout Great Salt Lake, and can be seen microscopically in virtually any water sample collected. However, marked differences in population density exist, since in the southern arm grazing animals, especially *Artemia salina*, consume vast amounts of *Dunaliella* and keep the water relatively clear, whereas in the northern arm

Table 3. *Effect of salinity on growth of Dunaliella enrichment cultures grown at -307 bars*

For experimental methods see text. Inoculation of culture media (over -300 bars), 11 to 12 June 1974. Transfer of successful cultures on 2 August to equivalent water potential. Test for optimum water potential begun on 30 September. Time of incubation, 24 days. Experiments were performed in duplicate from duplicate enrichments, but as both cultures gave similar results only one is given. Counts after 1 month, when total growth was higher, showed the same picture as that given here.

Culture	Habitat	Total dissolved solids (% w/v)	Growth ($10^{-5} \times$ algae/ml) at stated water potential of:		
			over -300 bars	-275 bars	-158 bars
76-1	Pothole Spring	9.2	2.0	4.1	6.4
76-6A	Spring Bay	29.6	2.6	5.5	7.0
79-2	Rozell Point*	32.8	4.2	7.9	10.9
79-3	Rozell Point†	32.8	0.5	1.7	1.8
80-1	Rozell Point†	32.8	0.23	0.45	0.88
80-2A	Rozell Point†	32.8	0.45	0.73	1.3
80-2B	Rozell Point†	32.8	1.5	2.6	3.7

* Planktonic material: inshore Great Salt Lake water.

† Benthic material: visibly green algal mats collected from a fossil algal bioherm submerged in an offshore location.

grazing animals, although present, are fewer in number, and the *Dunaliella* populations are correspondingly higher. The northern arm of Great Salt Lake is characterized by the brilliant red coloration of the water (Zahl, 1967), due at least in part to the red-coloured *Dunaliella*. In the southern arm, it appears that *Dunaliella* are always green, but in the northern arm the red phase predominates. These two colour phases have apparently been distinguished by some workers as the species *Dunaliella viridis* and *Dunaliella salina*, but no clear-cut distinctions except colour were apparent, and in culture the red phase always became green, even in medium containing saturating concentrations of sodium chloride. *Dunaliella* is not only present in the plankton; as a palmeloid form it is found coating rocks and other solid substrates throughout the lake.

Enrichment cultures

Because the detailed studies on isolate 123-4 (see below) indicated that the alga was not optimally adapted to the salinity from which it was obtained, a series of enrichment cultures were set up using inocula from northern lake locations. The northern arm is continuously saturated with NaCl, and has presented a relatively stable habitat (as far as salinity is concerned) since 1959. Cultures were prepared at different salinities from 0.87% (w/v) NaCl to 30% (saturated), corresponding to water potentials from -7 to greater than -300 bars. A wide variety of algae were obtained at the lower water potentials. At the highest water potential, (over -300 bars), only *Dunaliella* was obtained. After the cultures from over -300 bars had developed well, transfers were made, and from these transfers inocula were obtained for testing the effect of water potential on growth. Three salinities were used, corresponding to -158 bars, -275 bars and saturated (over -300 bars). All of the cultures grew better at -58 bars and showed progressively poorer growth as the salinity was raised (Table 3). Thus, none of these cultures was optimally adapted to the salinity of the enrichment culture from which it originated. Since the salinities of the natural habitats were somewhat higher than those at which the enrichment cultures were developed, it can be concluded that the *Dunaliella* populations from the northern arm of Great Salt Lake, although able to grow at the salinities present, are not optimally adapted.

Fig. 1

Fig. 1. Effect of NaCl concentration on growth of *Dunaliella* strain 123-4. Growth at: ▲, 6 days; ●, 10 days; ■, 17 days.

Fig. 2

Fig. 2. Effect of NaCl concentration on light-stimulated $^{14}\text{CO}_2$ uptake by *Dunaliella* strain 123-4.

Effect of salinity on growth and photosynthesis of culture 123-4

As noted in Methods, culture 123-4 was isolated from Great Salt Lake brine with a salt concentration of over 25 % (w/v) NaCl, and the alga was cultured in a medium containing about 25 % (w/v) NaCl, with periodic transfers, for three years before the present experiments were begun. Figure 1 shows the effect of salinity on growth. A broad salinity optimum exists, ranging from about 8 to 17 % (w/v) NaCl, but growth is poorer at 20 % (w/v) NaCl. Thus, even though the culture was isolated from 25 % (w/v) NaCl and maintained throughout at that salinity, its optimum salinity was lower. Experiments were also performed to measure the effect of salinity on $^{14}\text{CO}_2$ uptake in the light. A somewhat sharper optimum was found, between 10 and 15 % (w/v) NaCl, but this is in the same range as the optimum found for growth (Fig. 2); photosynthesis was much less at NaCl concentrations similar to those at which the organism was isolated and cultured.

Effect of salinity on photosynthesis by a natural population

Because culture 123-4 was not optimally adapted to the salinity of its original habitat, an experiment was set up to determine the salinity optimum of a natural population of *Dunaliella* from the north arm of Great Salt Lake. Material was collected and returned to Madison, where the experiment was done 2 days later. The water collected was visibly red; microscopic examination revealed low densities of the red phase of *Dunaliella* and fairly large numbers of rod-shaped bacteria. Because the density of algae was low, it was necessary to concentrate the cells by centrifugation at 15000 g for 30 min. The bright pink concentrate (containing both *Dunaliella* and pigmented bacteria, probably *Halobacterium*) was then diluted with 30 ml synthetic culture medium made up to various water potentials in 70 ml serum vials. After 15 min for equilibration, radioisotope was added. Because of the low cell densities, it was necessary to incubate overnight to obtain sufficiently high radioactivity in the samples. Each sample was filtered through a glass-fibre membrane filter (it was impossible to pass the liquid through a cellulose acetate filter), and the filter was washed with water of the same water potential and counted in a liquid scintillation counter.

The salinity optimum of the natural population found in this experiment was even lower than that of the culture, being about -28 bars, although significant photosynthesis occurred at salinities up to saturation (over -300 bars). It is not clear why the natural population

(having developed in northern arm brine which has remained saturated with NaCl since the late 1950s) should have a lower optimal salinity than the culture, but this natural population also is not optimally adapted to the salinity at which it is living.

DISCUSSION

The three types of experiments presented above show that *Dunaliella* from brine containing saturating concentrations of NaCl is not optimally adapted to this salinity, even though it is able to grow and photosynthesize under these conditions. Enrichment cultures using brine from the north end of Great Salt Lake showed that *Dunaliella* can be routinely cultured from water of this salinity in medium of the same salinity, but the optimum salinity of the enrichment cultures is lower (about 15 %, w/v, NaCl). A culture obtained from brine of 25 % (w/v) NaCl, and maintained for three years with successive transfers at this salinity, had an optimum salinity for growth and photosynthesis of 10 to 15 % (w/v) NaCl. A natural population from the north end brine was also not optimally adapted to saturated salt, even though the alga had developed in water which has not dropped below saturated NaCl since the late 1950s. Gibor (1956) isolated *Dunaliella* from a solar-salt pond and found an optimum salinity of 10 to 15 % (w/v). Johnson *et al.* (1968) isolated a culture from a similar solar-salt pond into a medium containing about 22 % (w/v) NaCl, but found that the optimum salinity for the culture was between 5 and 12 % (w/v) NaCl. Van Auker & McNulty (1973) found an optimum for growth of their *Dunaliella* culture from Great Salt Lake at 19 % (w/v) NaCl, higher than that reported in the present paper but still less than saturation. The conditions used by these workers may have permitted better growth at higher salinities than in the present work, since they took special care to use CO₂-enriched air.

Although these results suggest that it has been impossible for *Dunaliella* through evolutionary processes to adapt optimally to saturated salt, no other algae, eucaryotic or blue-green, seem able to compete with *Dunaliella* at high salinities, since other algae are rare (or absent) in the saturated brine and never appear in enrichment cultures at these salinities – although they grow readily in the same medium at lower salinities. Thus it appears that saturated salt presents a physico-chemical limitation which it has not been possible for most algae to overcome by evolutionary changes.

The effect of water potential on *Dunaliella* can be compared with the effects previously reported for other algae. Living in soil, the alga *Cyanidium caldarium* has a limiting water potential of about –80 bars (Smith & Brock, 1973). *Microcoleus vaginatus*, a blue-green alga from a desert crust, was found to have a limiting water potential of –18 to –28 bars (Brock, 1975*a*), and the coccoid green alga *Trebouxia* sp. from a variety of aerial and terrestrial lichens had limiting water potentials of –28 to –145 bars (within the lichen thallus the limiting water potential was lower, but this was thought to be an effect of the fungus; Brock, 1975*b*). Since *Dunaliella* grows and photosynthesizes optimally at –100 to –150 bars and is able to grow well at water potentials of over –300 bars, its ability to grow at low water potentials far exceeds that of any other alga so far studied.

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