SURVIVAL OF BLUE-GREEN ALGAE UNDER PRIMITIVE ATMOSPHERIC CONDITIONS*

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Abstract. Conditions on the primordial Earth are reviewed and information on pertinent microfossils and primitive microorganisms presented. A series of simulated pre-Cambrian environments are set up and 8 strains of blue-green algae are tested under 7 different anaerobic, mildly reducing atmospheres. Of 61 cultures tested, 12 showed growth, 32 survived and 17 died. Growth was measured spectrophotometrically. Microscopic examination failed to show any gross morphological changes in the experimental cultures. It is concluded that certain strains of blue-green algae have retained their ability to survive or grow under a primordial atmosphere.

1. Introduction

It is now well established that the atmosphere of the primitive Earth was reducing in nature and probably composed largely of nitrogen, ammonia, methane and water vapor. This primitive atmosphere may have existed, at least in part, up to 1.5 to 2.0×10^9 yr ago (Rutten, 1962). During the early pre-Cambrian there was a gradual oxidation of the reducing atmosphere caused by the molecular oxygen derived from both photodissociation of water vapor and photosynthesis.

Schopf and Barghoorn (1967) unearthed alga-like microfossils from the early pre-Cambrian strata of South Africa. These microfossils, which are 3.1×10^9 yr old, predate the rise of the modern oxidizing atmosphere by at least one and possibly two billion years. In reporting their findings, Schopf and Barghoorn postulated that the microfossils are either directly related to modern coccoid blue-green algae or that they are their evolutionary precursors. Siegel (1967) discovered a microorganism which has an affinity for high (25%) concentrations of ammonia and shortly thereafter found that it was identical to another microfossil found by Barghoorn and Tyler (1965). The fossil form was named *Kakabekia umbellata* and was discovered in the Gunflint chert deposit and is about two billion years old. The contemporary microorganism was named *Kakabekia barghoorniana. Kakabekia* cannot be readily classified among known groups of microorganisms but its ammonia affinity as well as its phylogeny suggest that the Earth had a reducing atmosphere long after the advent of the blue-green algae. Since the primitive Earth provided a reducing environment and since blue-green algae apparently evolved when such conditions prevailed, a series of seven experiments were performed to see if the algae could indeed survive and grow under a presumed primordial atmosphere.

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2. Materials and Methods

The following 8 cultures of blue-green algae were obtained from the collection of Dr George Claus:

A – *Synechococcus elongatus* Nag.
B – *S. elongatus* Nag. forma
C – *S. parvus* Mig.
D – *S. lividus* Copeland
E – *Synechosystis salina* Wislouck
F – *Anabaena variabilis* Kutz. f. *tenuis* Popova
G – *Gleothece rupestris* (lyngb.) Born. var. *tepidatriorum* (A. Br.) Hansg.
H – *Gleocapsa alpicola* (lyngb.) Born.

The algae were cultured in 13 × 100 mm glass culture tubes using Kratz and Myers (1955) 'C' medium. The environmental chambers used were 4L 'Anaero-Jars'; glass jars with aluminium screw type caps, rubber seals, pressure and vacuum gauges and needle valves. The algae were subcultured from the stocks aseptically into the tubes in duplicate, one serving as the experiment and one as the control. The experimental cultures, consisting of 5–8 different species of the algae listed above, were placed in the chambers and the matching controls were kept in normal air. The chambers were evacuated and flushed several times with pure nitrogen until an atmosphere of 99.9+% nitrogen was obtained. The chambers were then evacuated again and filled with the desired mixture of gases on a volume basis. All cultures were incubated at 24°C, 25 cm from a 75 W continuous light source (fluorescent). Growth, or the lack of it, was ascertained from turbidity change with a Bausch and Lomb Spectrophotometer at 540 nm. The cultures were examined microscopically to determine if any morphological changes occurred.

Results

A total of 61 cultures were tested under 7 different reducing atmospheres. At the termination of each experiment the cultures were divided into 3 classes: those that (i) died, (ii) survived, (iii) exhibited significant growth. Significant growth is defined as an increase of 0.5 Absorbance units during the time period under study (up to 21 days). Survival or stagnation is defined as no significant growth and death as visible and marked dissolution of the cultures. The spectrophotometric readings were cross-checked with microscopic examinations for culture density and cell size. Table I shows the per cent compositions of the atmospheres used, the number and types of cultures examinated, the duration of each study and the per cent and types of cultures that died, survived or exhibited growth.

3. Discussion

The results indicate that at least 8 strains of blue-green algae have some degree of tolerance toward anaerobic, mildly reducing atmospheres. This not surprising in light of the presumed phylogenetic history of the blue-green algae.

TABLE I

Atmospheres:	1	2	3	4	5	6	7
Percent composition	NH_3 – 10 CH_4 – 20 CO_2 – 30 N_2 – 30 H_2 – 10	NH_3 10 CH_4 – 30 CO_2 – 60	NH_3 – 5 CH_4 – 35 CO_2 – 20 N_2 – 40	NH_3 – 7 CH_4 – 13 CO_2 – 50 N_2 – 30	N_2 – 40 CO_2 – 30 CH_4 – 10 H_2 – 20	N_2 – 50 H_2 – 30 CO_2 – 20	N_2 – 50 CO_2 – 20 CO – 20 H_2 – 10
Number of cultures	9	12	5	8	9	6	12
Time in days	21	21	6	15	13	14	21
Types[a] and percent:							
death	11 D	58 BCDEH	40 AB	25 FH	22 EH	33 AB	8 H
survival	78 ABCEFH	33 ACE	40 EF	50 ACDE	66 ABCEFH	33 AC	50 ABCEF
growth	11 C	9 A	20 C	25 CE	12 C	34 AH	42 CFH

[a] For an explanation of the letters, see key in the text.

Under an atmosphere containing 50% CO_2 (No. 4), all the cultures survived and some showed growth. This atmosphere might be assumed to represent a stage in the evolution of life on Earth when presumably high partial pressures of CO_2 had been produced by anaerobic fermentation but the CO_2 had not yet been photosynthetically fixed by bluegreen algae or other phototrophic organisms. The partial pressure of CO_2 in this experiment was 1700 times greater than the modern atmospheric level. It is significant that the algae survived and also exhibited growth under such conditions.

Seckbach (1969) has investigated the survival of higher and lower plants under atmospheres of 100% CO_2. Only algae among the oxygen producing photosynthetic organisms survived the CO_2 treatment. The eucaryotic, acidophilic, thermophilic *Cyanidium caldarium* did well under CO_2 but the blue-green algae *Nostoc* and *Anabaena* could not be adapted to high concentrations of CO_2. Seckbach also stated that the blue-green algae *Synechococcus lividus* and *Mastigocladus laminosus* did not grow in pure CO_2 although *Cyanidium* did quite well. The results reported here indicate that *Anabaena variabilis* could not survive an atmosphere containing 50% CO_2 which seems to substantiate Seckbach's work. The cultures of *Synechococcus lividus* used in this study survived but did not grow under 50% CO_2 and died under 60% CO_2 (No. 2), an atmosphere containing no nitrogen. These results also agree with Seckbach's findings.

Addition of ammonia as a gas results in immediate solubilization into the liquid media forming NH_4^+. Probably there were never large amounts of free ammonia in the Earth's atmosphere after the formation of the primitive seas. The work of Siegel and Siegel (1970) has shown that *Kakabekia* certainly has an affinity for ammonia rich environments but the blue-green algae used in this study could not withstand the extremely alkaline (pH ca. 12) conditions that *Kakabekia* are reported to inhabit. Still, the algae were capable of tolerating atmospheres of up to 10% ammonia initial gas content.

High partial pressures of neither carbon monoxide or hydrogen were detrimental to the growth and survival of the algae. One experiment was run without any nitrogen (No. 2) but the algae in that experiment had the highest death rate (58%) of the whole series.

Although the above series of experiments gives only an indication of the phylogenetic antiquity of the blue-green algae, it appears that the group has maintained its tolerance for anaerobic, mildly reducing environments that were characteristic of the primitive Earth.

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