Characteristics of Marine Blue-green Algae with Uric Acid as Nitrogen Source

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SUMMARY

Two types of growth response were found with several species of marine blue-green algae when uric acid was sole nitrogen source for growth. For some species the cell type produced was typical of a blue-green alga and the growth rate was like that with other nitrogen sources. The second cell type was atypical in pigmentation. The organisms had a low nitrogen and ash content and the growth rate was much impaired. The cell type seen with uric acid is considered to be probably a minimum for sustained growth of a blue-green alga. Lipid accumulation was not a characteristic of the nitrogen-deficient condition, rather a carbohydrate reserve of a poly-glucose type is indicated. The growth data presented lead to the hypothesis that two different pathways for uric acid utilization may exist in blue-green algae, one probably a normal uricase type and the other a non-specific attack, possibly peroxidative.

INTRODUCTION

Uric acid has been reported to be utilized as sole nitrogen source for growth for several algae (Droop, 1955; Miller & Fogg, 1958) and might also be an important organic nitrogen source in marine environments, particularly inshore areas. While examining the mechanism of uric acid utilization by several marine species of blue-green algae we found two distinct growth responses. Some species of blue-green algae (e.g. *Plectonema terebrans*) gave a reasonable growth rate with uric acid as compared with nitrate, and had normal pigmentation. With other species (e.g. *Agmenellum quadriplaticum*) the growth rate with uric acid was much lower than with nitrate and the organisms looked as if they were suffering severe nitrogen limitation. We have examined the growth, elementary analysis and pigmentation of the cell type this produced, which appeared to represent an unrecognized, unusual and stable physiological state for a blue-green alga.

METHODS

The organisms used, except *Phormidium persicinum*, were those previously described by Van Baalen (1962). *P. persicinum* was obtained through the kindness of Dr L. Provasoli; we are indebted to Dr F. Drouet for identification of the cultures.

The growth medium used was ASP-2 (Provasoli, McLaughlin & Droop, 1957) with the following modifications: phosphate increased 10 times and nitrate 20 times; initial pH 8.2, S-3 and vitamin B₁₂ added only when needed. For the uric acid medium nitrate was omitted and 0.03% uric acid, sterilized by filtration (Morton

filter apparatus, Corning no. 83990) was added. The uric acid was dissolved by adding tris buffer and gentle heating. Allantoin and urea were also sterilized by filtration.

Growth experiments were done in large test tubes (50×400 mm.) incubated in illuminated, thermostatically controlled water baths designed after Myers (1950). Continuous culture of *Agmenellum quadriplaticum*, strain PR-6, was done in apparatus like that described by Myers & Clark (1944). Lighting was by one 13 W. fluorescent lamp inserted in the centre of the chamber. Incubation was at 39° . The chamber was operated manually, one-half the volume of culture was drained and replaced with fresh medium every 2 days. Carbon dioxide was supplied as 1% (v/v) CO_2 in air. Other details of growth conditions are noted in Results.

Uric acid was determined by using a uricase preparation (Worthington Biochemicals, Freehold, New Jersey, U.S.A.). Dry-weight determinations were done by filtering the material on tared Millipore filters, rinsing with distilled water, and drying at 75° .

The chemical analyses of organisms were done by Huffman Microanalytical Lab., Wheatridge, Colorado, U.S.A.

The spectra of whole organisms were measured on a Carey 14 Spectrophotometer (Applied Physics Corp., Monrovia, California, U.S.A.) with Lucite plates ($\frac{1}{8}$ in., no. 7328) inserted in the cuvettes after the method of Shibata (1958). We are indebted to Dr J. Myers for the use of the plates and the Carey Spectrophotometer. Other work on spectra was done with a Beckman DU Spectrophotometer.

Cell extracts were made using a Branson Sonifier, Model LS-75 (Stamford, Connecticut, U.S.A.). Glucose was identified and determined by using 'Glucostat' (Worthington Biochemicals) and by paper chromatography.

RESULTS

Growth responses to uric acid

Table 1 gives yields of nine marine isolates of blue-green algae grown with uric acid as sole nitrogen source. Two of the organisms, *Plectonema terebrans* strain Jamaica, *Lyngbya Lagerheimii* strain Mont, grew well and gave normal pigmentation. The other organisms which grew were bleached and showed a strong yellow coloration. The growth of strain Jamaica was followed further for comparison with *Agmenellum quadriplaticum* strain PR-6. Strain Jamaica grew with allantoin, urea or nitrate as nitrogen source with yields of organism of: mg. dry weight/20 ml. medium in 72 hr. of 8.3, 9.5 and 6.9, respectively. Strain PR-6 was selected for further study; the data following will deal with it.

Growth of Agmenellum quadriplaticum strain PR-6

Figure 1 presents the growth and uric acid utilization of *Agmenellum quadriplaticum* strain PR-6. The specific growth rate constant k in $\log_{10}$ units/day with uric acid as sole nitrogen source was 0.14; with nitrate under the same conditions the value of k was 2.2, and on urea 2.4. Strain PR-6 did not grow with allantoin, adenine, guanine, xanthine, hypoxanthine, uracil, 5-methyl-cytosine, cytosine or thymine as sole nitrogen source; nor when combined nitrogen was omitted from the medium. An uninoculated tube of uric acid medium prepared, incubated, and

treated in the same way as a growth run showed no evidence of uric acid decomposition. Apart from a slight increase in size of organism no microscopic differences between uric acid-grown and nitrate-grown organisms of strain PR-6 were observed.

Table 1. *The growth of certain blue-green algae with uric acid as sole nitrogen source*

Organism (stock designation in parentheses)	Yield of organism (mg. dry weight/20 ml. medium in 11 days)	Uric acid used (mg.)
Incubation at 39°, 600 ft.-c., fluorescent		
<i>Coccochloris elabens</i> (A-1)	5.9	1.5
<i>Microcoleus chthonoplastes</i> (Ba-1)*	14.4	3.3
<i>Anacystis marina</i> (6)	4.6	2.2
<i>Oscillatoria Williansii</i> (Mev)*	20.2	1.8
<i>Plectonema terebrans</i> (Jamaica)	41.2	6.0
<i>Agmenellum quadriplaticum</i> (PR-6)	24.0	6.0
Incubation at 30°, 400 ft.-c., fluorescent		
<i>Lyngbya lagerheimii</i> (Mont)	22.6	3.8
<i>Phormidium persicinum</i>	0.0	0.0
<i>Plectonema terebrans</i> (Cal Sp31)	0.0	0.0

* Growth very gelatinous.

Fig. 1. Growth (○) and uric acid utilization (×) by *Agmenellum quadriplaticum* strain PR-6. Temperature 39°; 600 ft.-c., fluorescent light.

*Elementary analysis of Agmenellum quadriplaticum strain PR-6
grown on uric acid and nitrate*

Table 2 presents the results of chemical analyses on samples of washed and dried uric acid-grown and nitrate-grown organisms of *Agmenellum quadriplaticum* strain PR-6. The R-value and percentage composition of protein, carbohydrate and lipid were calculated after the method of Spoehr & Milner (1949). The heat of combustion and R-value for uric acid-grown organisms were, as expected, low as compared to nitrate-grown PR-6 organisms and as compared to Spoehr & Milner's values for

Chlorella. A striking feature of the protein, carbohydrate, lipid calculation, even though admittedly an approximation, was the high carbohydrate value of the uric acid-grown organisms. The opposite effect was seen by Spoehr & Milner, namely, with *Chlorella pyrenoidosa* (Emerson) low nitrogen produced a high lipid content.

Table 2. *Cell analyses of Agmenellum quadriplaticum strain PR-6*

	Uric acid-grown organisms		Nitrate-grown organisms	
Carbon (%)	43.58	43.20	46.64	46.25
Hydrogen (%)	6.40	6.54	6.58	6.49
Residue (C-H) (%)	3.6	4.4	7.6	6.4
Nitrogen (%)	2.69	2.90	9.70	9.61
R-Value	32.8	—	37.3	—
Protein (%)	18	—	64	—
Carbohydrate (%)	74	—	27	—
Lipid (%)	7	—	9	—
4H _C	4.38 kcal./g	—	5.03 kcal./g.	—

A hot water extract of uric acid-grown PR-6 organisms gave no indication of a large soluble carbohydrate concentration. Upon hydrolysis of the residue from the hot water extract with 2N-H₂SO₄ for 6 hr. a strong positive test for glucose was found. Quantitative estimation showed that about 40 % of the cell dry weight could be accounted for as glucose; no other sugars were detected. Evidently a poly-glucose material is a main reserve product of uric acid-grown organisms. We have not further examined the nature of the polymer. It is clear, at least with strain PR-6 and perhaps it is a valid generalization for blue-green algae, that nitrogen deficiency does not predispose towards lipid accumulation. The same conclusion was tentatively reached by Fogg (1956) and Collyer & Fogg (1955). Calculation from the cell yield of organism in mg. dry weight shown in Fig. 1 and from the nitrogen content by analysis in Table 2 indicates that uric acid-grown strain PR-6 organisms consume on the average only one-third of the nitrogen of uric acid.

Pigment characteristics of Agmenellum quadriplaticum strain PR-6 on uric acid

Figure 2 presents three types of measurements of the pigment characteristics of *Agmenellum quadriplaticum* strain PR-6 grown with uric acid. There was slight variation in the curves depending on whether the organisms were batch grown or harvested from the continuous culture chamber, due we believe to slight impurities in the uric acid which can be used as nitrogen sources. By using the calculations of Myers & Kratz (1955) we find 0.29 % chlorophyll, 0.48 % phycocyanin, and 0.5 % carotenoid, for the curves shown. There is some uncertainty about the reliability of the values for phycocyanin. We are not presently prepared to make the statement that uric acid-grown cells of PR-6 contain no phycocyanin. Other blue-green algae we have examined had still lower % chlorophyll/mg. dry-weight values and even slighter indication of any phycocyanin. The data do reflect the fact that chlorophyll is more important for survival and sustained growth under suboptimal conditions. The carotenoid value is one-half the lowest value obtained by Myers & Kratz (1955) with *Anacystis nidulans*.

Pigment recovery

Figure 3 shows the results of a typical pigment recovery experiment, when washed uric acid-grown *Agmenellum quadriplaticum* strain PR-6 was supplied with ammonia, the required ions, and vitamin B₁₂. When the organisms were suspended in 1.8% NaCl + 0.16M-tris buffer (pH 8.1) + 1 mg. NH₄Cl/ml. and incubated in the light, only a small increase of pigment was seen. By visual observation of pigment

Fig. 2. Pigment curves for uric acid-grown *Agmenellum quadriplaticum* strain PR-6. Curves represent the extract of 2 mg. dry wt. organism/ml.; 1.0 cm. light path. ×, opal glass; ○, organism broken by Branson sonifier; ●, 80% acetone. Cell source continuous culture chamber, population density at harvest, equivalent to 0.9 mg. dry wt. organism/ml.

recovery we found that, in addition to the above materials, the following ions were also essential, all added at the concentration as in medium ASP-2: Mg²⁺, SO₄²⁻, FeEDTA, K⁺, PO₄³⁻.

Pigment recovery did not take place when helium or air scrubbed with KOH was passed through the tubes. Recovery was normal with 1% (v/v) CO₂ in nitrogen.

In the first few hours a rapid uptake of ammonia occurred and very little pigment synthesis was evident until after about 7 hr. The 2–3 hr. pigment curve characteristically showed a slight decrease in the 600–700 mμ region.

The greatest degree of recovery can be seen in the phycocyanin area of the curve. The time course for pigment recovery could be varied $\pm 30\%$ depending on the concentration of organisms and the light intensity used. It is of interest that, as shown in these recovery experiments and also in the cell analyses, uric acid-grown organisms in addition to nitrogen deficiency also had a mineral deficiency.

Fig. 3. Recovery curves, conditions: 33° , 400 ft.-c. tungsten. Cells from continuous culture chamber, washed and suspended in recovery medium. Curves are for whole organisms taken with opal glass, Carey 14 Spectrophotometer, *Agmenellum quadriplicatum* strain PR-6. Lower curve 0 time, middle curve after 10 hr. incubation, upper curve after 13 hr. incubation.

DISCUSSION

The following points about the blue-green algal type seen here when uric acid is sole nitrogen source may be made. As quoted by Fritsch (1952) nitrogen chlorosis of blue-green algae in natural conditions is commonly seen, the implication being that growth is not occurring. The data presented here show that nitrogen deficiency results in a stable physiological state and that continued and reproducible growth can be maintained under these conditions experimentally and probably under natural conditions. It seems probable that uric acid-grown *Agmenellum quadriplicatum* strain PR-6 represents a minimum condition for growth in a blue-green alga. We do not consider that the low growth rate and extreme nitrogen deficiency manifested by strain PR-6 while growing on uric acid is directly related to uric acid but rather to the very limited ability of the organisms to degrade it to a useful nitrogen source. We have searched for other nitrogen sources that would produce a similar effect but have found none. The possibility that uric acid or a product derived from its breakdown is acting as an inhibitor of some stage in nitrogen assimilation seems remote. We have found no evidence that uric acid, allantoin,

alloxanic acid, parabanic acid or oxonic acid (Brandenberger, 1954) affect strain PR-6 when nitrate is the nitrogen source.

A comparison of the growth data for *Agmenellum quadriplaticum* strain PR-6 and the *Plectonema terebrans* strain Jamaica growing on uric acid leads to the hypothesis that there are two different pathways for uric acid breakdown. We are tempted to ascribe to *P. terebrans* strain Jamaica and to *Lyngbya Lagerheimii* strain Mont a normal uricase, uric acid $\rightarrow$ allantoin $\rightarrow$ urea system. For *A. quadriplaticum* strain PR-6 and organisms which behave similarly, a non-specific peroxidase activity is a possible explanation (Canellakis, Tuttle & Cohen, 1954).

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