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THE HETEROTROPHIC GLUCOSE UPTAKE POTENTIAL OF THREE MARINE DINOFLAGELLATES

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The heterotrophic potential of the marine dinoflagellates *Prorocentrum micans* Ehr., *Gymnodinium splendens* Lebour. and *Gyrodinium fissum* (Lev.) was studied in unialgal cultures as net assimilation of ^{14}C-glucose during light and dark incubations. The heterotrophic potential of algal cells was highest in the early stages of culture growth. Light and dark incubations did not differ significantly in heterotrophy. *G. splendens* had the highest heterotrophic potential according to both maximum velocity per cell and the half-saturation constant (K) of glucose. The kinetic parameters of all cultures indicated low affinity for glucose. Half-saturation constants (140—1000 μg glucose · l^{-1}) exceeded those usually measured in natural planktonic communities by one to three orders of magnitude. It therefore seems unlikely that measurements of the heterotrophic activity or potential of natural microplankton would be affected by these mixotrophic algae to any significant extent.

Index words: Marine dinoflagellates, algal heterotrophy, heterotrophic potential, glucose assimilation.

1. INTRODUCTION

Several fractionation studies have indicated that in measurements of the heterotrophic activity or potential of natural microplankton, the size fraction corresponding mainly to free-living bacterial cells (appr. 0.2—2 μm) is responsible for the major portion of heterotrophy (e.g. Williams 1970, Allen 1971, Berman 1975, Gocke 1975, Azam and Hodson 1977). Wright and Hobbie (1966) presented data on net assimilation of glucose and acetate by natural communities of bacterioplankton and phytoplankton. They postulated that in heterotrophic assimilation measurements at relatively low substrate concentrations (<300 μg · l^{-1}), bacteria completely dominate uptake because of their higher affinity for the substrate. Therefore it could be safely assumed that assimilation measurements with low substrate concentrations describe the heterotrophic metabolism of bacterioplankton. These results have also been supported by autoradiographical studies (e.g. Hoppe 1976).

However, recent literature on the physiology of several groups of phytoplankton has presented accumulating evidence of the heterotrophic capacity of these organisms. In particular nano- and picoplankters belonging to the Chrysophyceae, bluegreen algae (Cyanobacteria) and dinoflagellates, as well as microzooplankton species of Zoomastigophora, have been thought to utilize dissolved organic substrates in their nutrition.

Owing to the lack of sufficient experimental data, these considerations are still speculative with regard to natural conditions, in which concentra-

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tions of dissolved organic compounds of low molecular weight are in the order of micrograms per litre. Field evidence for active algal heterotrophy (McKinley 1977, Vincent and Goldman 1980) is scarce and partly ambiguous (for discussion see Tamminen 1984).

In this study, heterotrophic potential was measured in unialgal cultures of *Prorocentrum micans* Ehr., *Gymnodinium splendens* Lebour. and *Gyrodinium fissum* (Lev.). Three phases of culture growth were assayed with *P. micans* and two phases with *G. splendens* in order to evaluate the effect of the physiological state of cells on their heterotrophy. The heterotrophic potential of the algae was measured as net assimilation of ^{14}C—glucose in both light and dark incubations.

2. MATERIALS AND METHODS

The algae were cultured in a luminostat with a 14 + 10 hour photopperiodicity (light + dark). Light intensity was 8 000 lux and temperature 19°C. The cultivation medium is presented in Table 1. The cell density of each culture was determined microscopically at the time of incubation.

Incubations (18 ml) were performed in sterile 20 ml test tubes. The incubation time was 4 hours, and it was terminated by adding Lugol solution. Blanks were prepared by adding Lugol solution to test tubes before additions of radioactivity.

D—(^{14}C—U) -glucose (specific activity 396 $\mu Ci \cdot mmol^{-1}$, Techsnabexport, Moscow, USSR) was added into subsamples at final concentrations of 12.3, 123 and 1270 μg glucose $\cdot l^{-1}$. Each concentration was added in duplicate and a blank sample was used. After termination of the incubations, samples were filtered on 2.5 μm membrane filters (Synpor, Czechoslovakia). The filtrates were filtered on 0.45 μm membrane filters (Gelman, USA). All filters were washed with 15 ml of filtered sea water of salinity equal to that of the media.

Samples were measured with Mark II and Mark III liquid scintillation counters (Packard, USA) by the external channel ratio method. The scintillation cocktail consisted of a filter, 100 μl of H_2O and 10 ml of Reahim ZS-7A scintillation cocktail (Harkovskyi zavod himreaktivov, USSR). The measured dpm (disintegrations per minute) values were calculated into turnover times ($T = C \cdot t \cdot c^{-1}$, where C = added radioactivity, t = incubation time, c = measured radioactivity).

Table 1. Goldberg medium for algal cultivations (Kabanova 1961). The salinity of the medium was 26‰ and the pH 8.0.

KNO_3	202 mg
Na_2HPO_4	7.1 μg
$FeCl_3 \cdot 6H_2O$	270 μg
$MnCl_2 \cdot 4H_2O$	198 μg
$CoCl_2 \cdot 6H_2O$	238 μg
synthetic seawater	1.0 l

Kinetic parameters describing the uptake of glucose were calculated according to Wright and Hobbie (1965, 1966) for both <2.5 μm and >2.5 μm size fractions. Since the media contained no glucose, the sum parameter (K + S) reduced to K, the half-saturation constant for the substrate.

3. RESULTS

The uptake kinetics of different size fractions differed qualitatively from each other in all the cultures (Fig. 1). Measurements of the algal fraction (>2.5 μm) followed closely the linear transformation of Michaelis-Menten kinetics over the assayed concentration range. It was obvious, however, that the 1270 $\mu g \cdot l^{-1}$ addition exceeded the saturation concentration for bacteria, resulting in two different phases of glucose uptake. Below the 123 $\mu g \cdot l^{-1}$ addition, bacteria showed very significantly greater affinity for glucose than did algae, with K values in the order of only a few

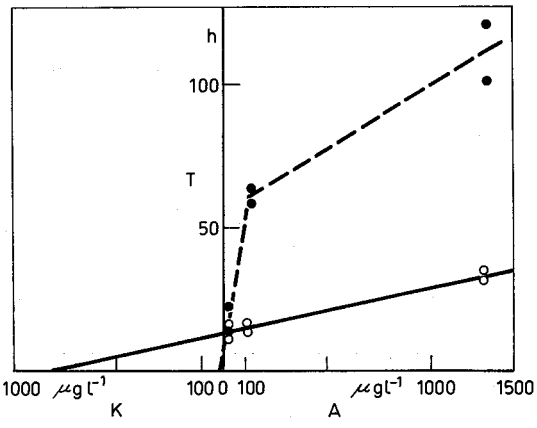

Fig. 1. Glucose uptake kinetics in different fractions of dark incubations of *Gyrodinium fissum*.

micrograms per litre. It was therefore concluded that the fractionation between algae and bacteria in the cultures had succeeded satisfactorily, and that the measured glucose uptake in the >2.5 μm fraction described the heterotrophic metabolism of algae.

The relationship between algal heterotrophy in light and dark incubations varied between 0.83 and 1.20, with a mean of 1.00 (Table 2). No stimulative or inhibitive effect of light on algal heterotrophy was thus observed. The heterotrophic potential (maximum uptake velocity of glucose, V) was transformed into per cell values to investigate the effect of the physiological state of cells on their heterotrophic potential (Table 2). It appeared that with both *P. micans* and *G. splendens*, when cultures of different ages were assayed, the heterotrophic potential decreased with age, being highest during the logarithmic growth phase of the cultures.

When different species were compared *G. splendens*, which grew in the lowest densities, had clearly the highest maximum uptake velocity per cell. Old *P. micans* cultures had the lowest heterotrophic potential values (Table 2).

4. DISCUSSION

Values of the half-saturation constant (K) are linear extrapolations of uptake measurements, when the so-called Lineweaver-Burk transformation is used in the calculations (see Fig. 1). When the extrapolation distance increases, the precision of the constant naturally decreases. This is reflected in the values in Table 2, as the half-saturation constant should be a more or less stable property of the culture — affinity for the substrate — so that the values in light and dark incubations would be expected to agree closely. However, the value of the constant shows reasonable variation in the experimental data. The smallest values — thus indicating the greatest affinity for glucose — were calculated for *G. splendens* cultures (140—240 $\mu g \cdot l^{-1}$).

Direct comparison between the results obtained from laboratory cultures and those from natural communities is meaningless, because the physiological state of the cells is not equal. The half-saturation constant (K) in natural communities is also a somewhat problematic parameter, as it is not in fact a constant, but necessarily a temporary weighted mean of different species, fluctuating during the course of succession within the community. Furthermore, the separation of the components of the kinetic sum value ($K + S_n$) obtained in the substrate uptake analysis of natural communities is laborious, and the sum value can usually be considered only as an upper limit for both parameters.

With these constraints in mind, the half-saturation constant can be used as a general indicator of the utility of the substrate for planktonic community. The K values in Table 2 are one to three orders of magnitude higher than those usually measured with natural microplankton (see for example Sepers 1977, Hoppe 1978, Kuznetsov and Romanenko 1979). This difference can hardly be attributed only to the effects of laboratory conditions.

Table 2. Kinetic parameters of glucose uptake in the algal fraction.

Species	Density	Light	V	V per cell	K	V (dark)
	$cell \cdot ml^{-1}$	%	$\mu g \cdot l^{-1} \cdot h^{-1}$	$pg \cdot h^{-1} \cdot cell^{-1}$	$\mu g \cdot l^{-1}$	V (light)
Prorocentrum micans	3040	0	57.6	18.9	1000	
P.micans	3040	100	50.2	16.5	530	1.15
P.micans	5880	0	37.2	6.3	290	
P.micans	5880	100	38.9	6.6	260	0.95
P.micans	8220	0	55.4	6.7	480	
P.micans	8220	100	60.8	7.4	620	0.91
Gymnodinium splendens	320	0	19.1	59.7	210	
G.splendens	320	100	15.9	49.7	140	1.20
G.splendens	600	0	17.5	29.2	240	
G.splendens	600	100	18.1	30.2	210	0.97
Gyrodinium fissum	6420	0	64.3	10.0	810	
G.fissum	6420	100	78.0	12.1	910	0.83
Mean						1.00

Therefore, it can be assumed that these mixotrophic dinoflagellates are likely to play only a minor role in the cycling of dissolved organic compounds of low molecular weight in nature. Our results are in agreement with the paradigm previously presented for the heterotrophy of natural bacterio- and phytoplankton (Wright and Hobbie 1966), as the glucose uptake kinetics of the dinoflagellates were qualitatively less efficient than those of both natural bacterioplankton and bacteria in the laboratory cultures. Concentrations of glucose — or of other components of the labile dissolved organic carbon pool — in natural water bodies hardly ever reach the range in which algal heterotrophic capacity would overcome that of bacterioplankton (hundreds of micrograms per liter), with the possible exception of extremely eutrophic conditions.

This conclusion of the quantitatively minor importance of dinoflagellate heterotrophy for the cycling of the labile fraction of dissolved organic carbon does not neglect the possible qualitative significance of heterotrophy for algal nutrition. It is here considered mainly with regard to the carbon cycle and the utilization of the single concentration method for determination of the heterotrophic potential of natural bacterioplankton communities. If substrate additions are in the range of those presented by Kuparinen et al. (1984), these experiments support the assumption that the measurements describe the heterotrophic metabolism of bacterioplankton.

LOPPUTIIVISTELMÄ

Merellisten dinoflagellaattien *Prorocentrum micans* Ehr., *Gymnodinium splendens* Lebour. ja *Gyrodinium fissum* (Lev.) heterotrofista potentiaalia mitattiin ^{14}C-glukoosin nettoassimilaation avulla valossa ja pimeässä. Leväsolujen heterotrofinen potentiaali oli korkeimmillaan nuorissa viljelmissä. Valo ei vaikuttanut heterotrofiaan. *G. splendens* osoittautui tehokkaimmaksi glukoosin käyttäjäksi sekä solukohtaisen maksimaalisen ottonopeuden että puolikyllästysvakion (K) perusteella.

Kaikkien viljelmien glukoosin käyttö oli tehotonta. Puolikyllästysvakiot (140—1000 μg glukoosia $\cdot$ l^{-1}) olivat kertaluokkia korkeampia kuin luonnon planktonyhteisöissä tavallisesti mitatut. On epätodennäköistä, että nämä miksotrofiset dinoflagellaatit vaikuttaisivat merkittävästi vesistöissä mitattaviin heterotrofisiin parametereihin.

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