THE USE OF *ESCHERICHIA COLI* AS FOOD SOURCE FOR PROTOZOA IN EXCRETION EXPERIMENTS : PRELIMINARY RESULTS

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Abstract

Quantification of dissolved organic carbon (DOC) egestion rates of marine protozoa is hindered by the uptake of the egested DOC by marine bacteria. Here, we present a new method to measure DOC egestion rates of protozoa by replacing the marine bacteria by *Escherichia coli* as food source. *Escherichia coli* is an enterobacteria that becomes instantaneously inactive upon addition to seawater. Thus, *E. coli* will not take up or release any metabolic products in spite of being alive. Indeed, we found no uptake or release of DOC by *E. coli* in seawater. Also, *E. coli* appeared to be a good food source for a ciliate, *Strombidium sulcatum*, and a small flagellate isolated from NW Mediterranean waters.

Key-words: carbon, bacteria, predation

Introduction

Dissolved organic matter (DOM) represents a major fraction (> 95%) of total organic matter in the ocean and has important short and long term implications for plankton trophic interactions and oceanic biogeochemistry (1). However, little is known about the characterization, transformation and absolute concentration of DOM in seawater (2). Recently, it has been reported that dissolved organic carbon (DOC) integrating this DOM seemed to accumulate in high concentrations in upper and biologically active waters during stratification periods (3). This net production of DOC originates from in situ biological production. Direct active or passive exudation from phytoplankton, sloppy feeding and excretion from protozoan and metazoan grazers, and cell lysis from viral infection have been suggested as sources for this DOC production.

The degradability of this surface DOC pool has been studied intensely over the past years (4). Apparently 50% of it is refractory DOC while the other 50% is semi-labile and degradable over the time scale of months (5). To some degree, the recalcitrant DOC is imported from allochthonous sources, but it may also be the result of transformations of labile compounds originating from autochthonous primary production. Tranvik (6) reported that DOC produced by flagellates was digested slowly by the bacterial assemblage. He suggested that flagellate bacterivory could contribute to the transformation of labile organic matter into more refractory forms.

Theoretical calculations indicate that small flagellates could ingest 10^4 - 10^5 bacteria per ml per day (7). Assuming a carbon content of 20 fg C bacteria^{-1} (8), and 20% of the ingested bacteria eliminated as DOC (6), we calculate that small flagellates could release 0.04-0.4 mg C l^{-1} d^{-1}. The DOC accumulation reported for the surface layer in the Mediterranean during the stratified period, 200 mg C l^{-1} over 6 months (3), is equivalent to about 1 mg DOC l^{-1} d^{-1}. Approximately 4 to 40% of this DOC could be produced by the stock of small flagellates. In spite of the possible importance of protozoan excretion on DOC dynamics in surface waters, there is a lack of reliable data on assimilation efficiencies and egestion rates of protists (9, 10). Information on the assimilation efficiencies of protozoa feeding on natural prey is essentially non-existent. This is due to the difficult task of recovering egested materials and added complications derived from bacterial uptake of the produced DOC.

Due to the consumption of egested DOC by the bacterioplankton, the use of live bacteria represents a problem in obtaining reliable DOC data. In order to circumvent this obstacle, we have developed a new method consisting on using *Escherichia coli* as food source for the protozoa instead of marine bacteria. *Escherichia coli* is an enterobacteria that becomes instantaneously inactive upon addition to seawater. Thus, the bacteria will not take up or release any metabolic products. However, E. coli will remain alive as opposite to the often used heat-killed bacteria. The use of live bacteria will avoid heat-killing (at 60°C for 4 h) natural assemblages and therefore the denaturalization of organic compounds that may affect DOC egestion rates. Although of non marine origin, E. coli is a gram-negative bacteria like most marine assemblages. Another advantage of using E. coli in our experiments is the absence of metabolic products derived from bacterial activity.

Material and methods

Escherichia coli (strain HB10B) was grown on Luria-Bertani medium at 37°C and with vigorous shaking. The culture, which had reached ~ 1 unit optical density at 600 nm, was centrifuged in 15 ml centrifuge tubes at 9000 rpm during 10 min. The supernatant was rejected and substituted by 0.2 μm filtered and autoclaved seawater. Each centrifuge tube was sonicated for 1 min to detach bacteria from medium particles, and centrifuged again. This procedure was repeated 4 times.

Uptake or release of DOC by E. coli

Centrifuged *E. coli* were added to two 1 l Erlenmeyers containing 400 ml of 0.2 μm filtered and autoclaved seawater to a final concentration of ~ 7 x 10^6 *E. coli*/ml. To one of the erlenmeyers, sterile D-glucose was added to a final concentration of ~140 mM. Both Erlenmeyers were incubated at 37 °C and with vigorous shaking.

Dissolved organic carbon samples were taken initially, at 1 h intervals for 10 h, and 21 h after setup. These samples were filtered on 0.2 μm Gelman Supor filters, which had been previously acid-washed (10% HCl) and rinsed with distilled water. The filtration was carried out on a glass Millipore filtration unit which had been ignited at 500°C for 4 h. The first 5 ml of the filtrate was rejected and the last 10 ml collected on 20 ml ignited Pyrex tubes, acidified to pH 2 with 2 N HCl, and stored at 5°C until DOC analysis. All samples were analyzed within a week of collection. Dissolved organic carbon was determined by high-temperature catalytic oxidation. Samples were sparged with an artificial air mixture (AGA, France) containing ≤ 0.1 ppm CO, CO_2 or hydrocarbons, and measured on a Shimadzu TOC-5000 instrument equipped with a high sensitivity catalyst. Dissolved organic carbon concentrations were calculated with the instrument software and a 4-(no D-glucose addition samples) or 2-point (D-glucose added samples) standard calibration curve made with potassium biphthalate. The coefficient of variation of duplicate injections was always < 2%.

Samples for bacterial enumeration were taken initially, every 3 h for 10 h, and 21 h after setup. These samples were fixed with 0.2 μm filtered formalin (4% vol./vol. final concentration), DAPI stained (11), and counted on an epifluorescence microscope.

Adequacy of E. coli *as food source for protozoa*

A 3 μm flagellate isolated from Northwestern Mediterranean waters and a 30 μm ciliate, *Strombidium sulcatum*, were fed *E. coli*. Samples were taken for counting S. sulcatum (enumerated on an inverted microscope by Utermöhl's (12) counting technique), and the flagellate and bacteria (counted on an epifluorescence microscope as described above).

Results

Uptake or release of DOC by E. coli

During the 21 h of incubation, DOC values were 130 ± 6 μM and 141.5 ± 4.9 mM in the Erlenmeyers containing *E. coli* and incubated without and with D-glucose, respectively. No accumulation or consumption of DOC ($n = 12$, $p < 0.0001$) took place during the incubations (data not shown).

Bacterial numbers were 7.7 ± 0.5 x 106 E.coli/ml for the 21 h of incubation and for both D-glucose added and not added Erlenmeyers. No bacterial growth ($n = 5$, $p < 0.0001$) took place during the incubations (data not shown).

Adequacy of E. coli *as food source for protozoa*

Figures 1 and 2 show the growth curves for the small flagellate and *S. sulcatum*, respectively. As the number of protozoa increased, there

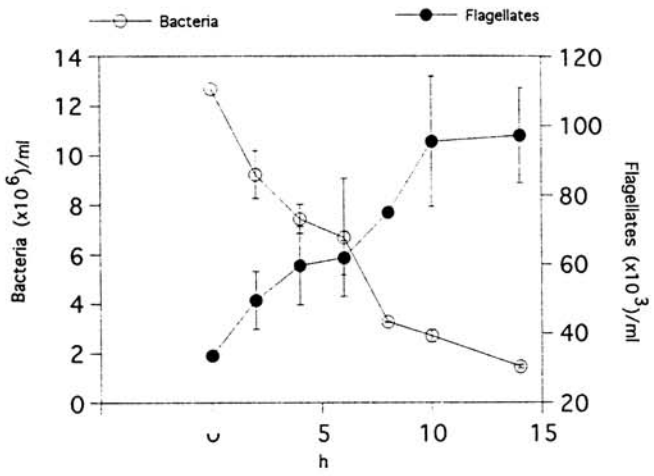

Fig. 1. Flagellate (x10^3) and bacteria (x10^6) per ml during the incubation period.

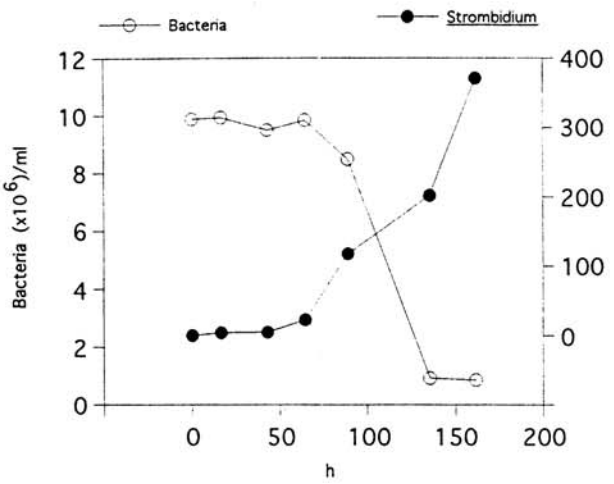

Fig. 2. *Strombidium sulcatum* and bacteria (x10^6) per ml during the incubation period.

was a decrease in bacterial numbers. A generation time of 7.5 and 10.1 h^{-1} was calculated for the flagellate and *S. sulcatum*, respectively. Generation time of the protozoa was calculated from regression of the linear portion of the increase in the natural logarithm of cell density during exponential growth of the organism, by applying the equation GT = ln2/S (S = slope of the regression line) (data not shown).

Discussion

These preliminary experiments indicate that *E. coli* is a good food source for the small flagellate and for *S. sulcatum*. In previous experiments, we had measured a generation time of 12.4 h^{-1} for *S. sulcatum* grown with marine bacteria. Thus, a generation time of 10.1 h^{-1} indicates that apparently *E. coli* is a better food source for the ciliate, probably due to their expected lack of metabolic activity. Indeed, our experiments confirmed a lack of metabolic activity, measured as production or uptake of DOC (D-glucose) by the bacteria in seawater.

In conclusion, these preliminary experiments confirm the adequacy of using *E. coli* as a model for measuring DOC egestion by protozoa.

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