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Measurement of bacterioplankton production in antarctic coastal waters: Comparison of thymidine and L-leucine methods and verification of labeling patterns

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Bacterioplankton are important components of marine pelagic ecosystems (Azam et al. 1983) and bacterioplankton production is an important sink for organic material in most of the ecosystems studied to date (Cole et al. 1988). Recently, questions have been raised about the quantitative significance of bacterioplankton production in carbon fluxes in polar waters (Pomeroy and Deibel 1986; Pomeroy et al. 1991; Huntley et al. 1991). To investigate the significance of, and factors controlling, bacterioplankton production in antarctic coastal waters, we measured bacterioplankton production using the ³H thymidine (Fuhrman and Azam 1982) and ³H L-leucine methods (Kirchman et al. 1985; Simon and Azam 1989). In view of the potential for metabolism of exogenous thymidine to complicate interpretation of incorporation data (Hollibaugh 1988), we performed experiments to verify the expected macromolecular labeling patterns. The results of those experiments, and a comparison of bacterial carbon production estimates calculated from thymidine and L-leucine incorporation rates are presented.

Experiments were performed with surface water collected at a station in the vicinity of Spume Island, about 3 kilometers from Palmer Station, Antarctica (64°48' S 64°06' W). This area was sampled from 15 October 1990 through 15 January 1991. Another sample was collected in the vicinity (64°50.2' S 64°5.7' W) on 20 August 1991. Near-surface water was collected in clean glass or plastic containers and immediately returned to the laboratory where it was held in a dark incubator at -1.0 °C.

Routine incorporation-rate measurements were begun by adding methyl-[³H]-thymidine (TdR, 81.0 uCi/nmol) or L-4, 5-[³H]-leucine (L-leu, 53 uCinmol) to triplicate 10.0 mL samples contained in sterile polystyrene culture tubes to final concentrations of 10 nanomoles. A subsample [5.0 mL] was removed from each tube immediately and filtered through 0.45 micrometers pore size Millipore filters (HAMF) as a blank. Filters were rinsed with seawater with chilled 5 percent (w/v) trichloroacetic acid (TCA). The remaining sample was incubated at -1.0 °C for 6-8 hours (time courses were linear over this interval) then filtered through HAMF. Filters were placed in scintillation vials, dissolved with ethyl acetate, and radioassayed using an LKB Rackbeta Model 1217 liquid scintillation spectrometer with Aquasol II as the scintillation cocktail. Incorporation rates were converted to bacterial biomass production rates using the factors given in Fuhrman and Azam (1982), Lee and Fuhrman (1987), and Simon and Azam (1989).
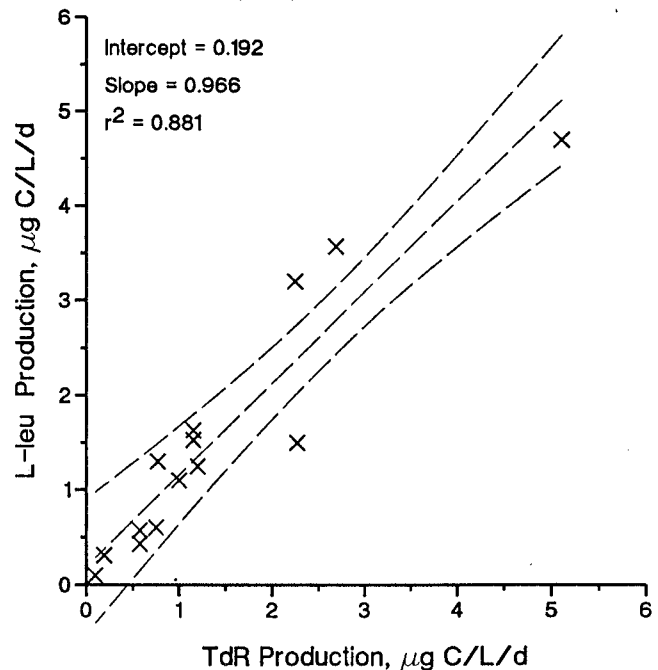
Comparison of bacterioplankton productivity measurements based on methyl-[³H]-thymidine incorporation vs. productivity measured in the same sample by L-4, 5-[³H]-leucine incorporation. The model 1 least squares linear regression line for the data and 95 percent confidence belts are also shown.

We examined the distribution in macromolecules of the incorporated ^{3}H in an experiment on 5 November 1990. Macromolecular fractionations were performed by acid-base hydrolysis of samples collected on 0.2 micrometer pore-size Nuclepore filters (NF) as described in Hollibaugh (1988). We also examined the size fractionation of incorporation by comparing the amount of label retained by 0.2 vs. 1.0 micrometer pore size Nuclepore filters.

The figure shows that most (92 percent) of the ^{3}H from TdR was incorporated into the DNA fraction, while most (88 percent) of the ^{3}H from L-leu was incorporated into the protein fraction. We obtained small, negative values of ^{3}H incorporated into the RNA fraction with both substrates. Since the amount of ^{3}H in the RNA (and DNA) fractions are calculated by subtraction, these values can be negative. We calculate that at least 78 percent of the ^{3}H from TdR was incorporated into DNA and that at least 88 percent of ^{3}H from L-leu was incorporated into protein.

Only a small fraction of the TdR and L-leu ^{3}H incorporation (3 and 11 percent, respectively) was associated with particles retained by 1.0 micrometer pore-size Nuclepore filters. Hence, TdR and L-leu were incorporated primarily by free-living bacterioplankton rather than bacteria attached to particles or by eukaryotic microorganisms. The chlorophyll concentration in this sample was 0.5 micrograms per liter and bacterial abundance wa 0.3 x 10^{6} cells per milliliter.

Bacterial carbon production calculated from TdR incorporation agreed well with bacterial carbon production calculated from L-leu incorporation over a large range (0.1 to 5.1 or 0.1 to 4.7 micrograms of carbon per liter per day, TdR and L-leu, respectively, figure 1). The slope and intercept of the regression line are not significantly different from 1 and 0, respectively (Student's t-test, $p < .0001$).

The results of this study indicate that either TdR of L-leu may be used to estimate bacterioplankton production reliably in antarctic coastal waters. However, there are advantages to using L-leu in waters of extremely low bacterioplankton productivity. L-leu is available at higher specific activities and more L-leu is incorporated per unit of bacterial carbon production, thus the detection limit for bacterioplankton productivity is lower with L-leu than with TdR.

Results similar to ours were reported by Bjornsen and Kuparinen (1991) for bacterioplankton populations from the Weddell Sea/Scotia Sea confluence. It is noteworthy that some of the experiments that lead to the widespread use of TdR for estimating bacterioplankton production were conducted in antarctic waters (Fuhrman and Azam 1980). Confirmation that the TdR method is reliable in antarctic waters, despite recent reports to the contrary (Karl et al. 1991), suggests that the extensive Research on Antarctic Coastal Ecosystem Rates Program TdR incorporation data set (Karl et al. 1991) can now be interpreted in terms of bacterioplankton production.

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