The temperature-substrate controversy resolved?

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ABSTRACT

We have postulated that bacterial growth and respiration are limited by substrate availability in the initial phase of temperate spring algal blooms when water temperature is near its annual minimum. Substrates for bacteria are low at bloom initiation, because phytoplankton release only a few per cent of their photosynthate directly. The early bloom is dominated by diatoms or other large phytoplankters that are directly available to zooplankton, but zooplankton are scarce initially and a significant part of the bloom sinks to the benthos before zooplankton have reproduced and are producing bacterial substrates through feeding and digestive processes. Experiments with natural bacterial communities and with pure cultures of marine bacteria suggest that increases in either substrate concentration or temperature result in increased bacterial growth. This will occur later in the spring bloom as either temperature rises, zooplankton produce bacterial substrates, or both. Thus, the timing and location of observations of bacterial activity may strongly influence the results and may have led to conflicting interpretations.

Introduction

For over 100 years it has been recognized that bacteria can grow and thrive at 0°C and below, yet for over half a century this observation was considered a curiosity and subject of few investigations outside of the food industry. However, with the acknowledgement that bacterial activities can play pivotal roles in ocean food webs, the appreciation that much of the oceanic productivity that produces fish occurs on continental shelves between 40° and 60° N in the Atlantic and Pacific Oceans and the Bering Sea, and most recently the recognition that any global warming effect would significantly raise temperatures in these regions, there has been greatly increased interest in the mechanisms that control bacterial growth at low temperature. Today, many groups worldwide are examining these issues. As often happens in science when a field is developing and a variety of approaches and techniques are used, questions and controversies arise. The subject of this paper is one such topic, the relationship between temperature and organic substrates for the growth of bacteria at low temperature.

Hypothesis and Implications

The general hypothesis can be stated as follows: Bacterial growth and respiration are limited by substrate availability when water temperature is near the annual minimum, whatever it may be. The hypothesis was developed and examined in a series of investigations conducted in Conception Bay, Newfoundland at latitude 48° N [10,13,12] and later in the Arctic [11,17,1]. In addition, the hypothesis was extended to subtropical

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waters [16]. In fact, it applies more generally, even to *Escherichia coli*. The temperature-substrate response of *E. coli* is nearly flat in its optimal temperature range, but generation times rise steeply as limiting temperature-substrate conditions are approached (Fig. 1).

There are two implications that can be derived from this hypothesis:

A. As documented by Pomeroy and Deibel [10] and Pomeroy et al. [13], under early spring bloom conditions at -1 to 0° C in Conception Bay, Newfoundland, the microbial loop functioned minimally. Only a small percentage (<5%) of the primary production was being consumed by the bacteria, whereas in other areas of the ocean or at other times in the areas examined, 20-60% of the primary production has been estimated to be consumed. Since there is a large area of the ocean that also develops spring blooms, this behavior may be considered a quantitatively significant phenomenon.

B. The second implication is that virtually all of the primary production in this initial period of the bloom is available directly to benthic metazoa. These spring blooms are dominated by diatoms, which can and do sink to the ocean floor. Jumars et al. [3] have made a strong case that sinking bloom algae constitute the major—and pulsed—source of organic matter for benthic animals. Thus the reduced microzooplankton grazing and resulting reduced activity of the microbial loop increases food availability to the benthos.

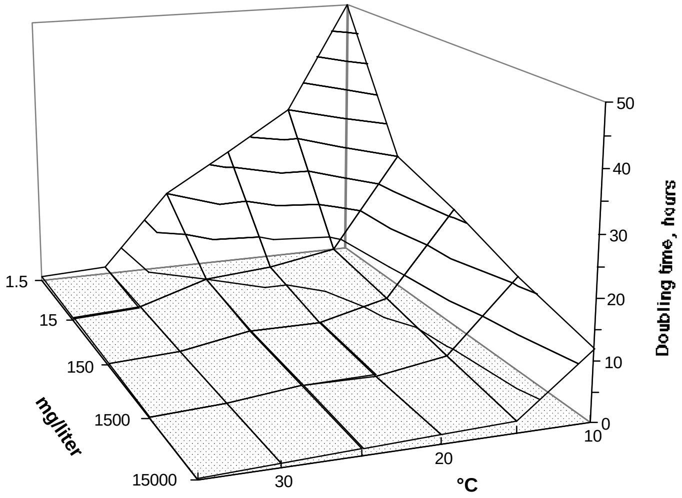

Fig. 1. Relation of growth rates of *E. coli* to substrate concentration and temperature (data from [16]).

Discussion

It would be unnatural, indeed upsetting, if the proposal presented above did not stimulate some controversy, and it has. A number of published studies dispute the hypothesis and a number have supported it. In this section we will discuss three major issues that have arisen and attempt to resolve them or point out areas that need further examination.

The first problem concerns the ability of bacteria to grow at low temperature. Microbiologists have provided abundant evidence that bacteria in culture and in nature can grow rapidly at low temperatures [7]. However, in all of these studies, very high concentrations of substrate have been used, generally on the order of grams per liter. In our culture studies we also found relatively rapid growth rates at 0°C, provided substrate concentration was high [17]. Similarly, Pomeroy and Deibel [10] and Pomeroy et al. [11] demonstrated that the respiration of natural populations, while minimal or unmeasureable in unamended samples, was substantial if amended with substrate, or it the temperature was elevated from the spring minimum temperature of -1 or 0° to +2 or 3.5°C. Nedwell and Rutter [1990] have provided evidence for a mechanism that may explain these data. They found that at temperatures approaching the minimum for growth of two psychrotolerant Antarctic bacteria, there was a decrease of specific affinity for substrate. Thus at low temperatures, higher substrate concentrations are required for growth than at higher temperatures. Figure 1 presents a graphical example of this statement. We find this same relationship in many cultures of marine bacteria. The problem appears to be not the lack of growth per se at minimal temperatures, but the lack of sufficient substrate in the natural environment.

Studies in cold sediments [9] and in sea ice [4], in which bacterial activities approach those in warmer waters, have also been used to dispute the hypothesis. However, these environments contain relatively high natural substrate concentrations and thus are not comparable to the conditions in the water column (It should be noted that the authors cited do not make this claim.).

A second problem involves the source of substrates for bacteria. As reviewed by Pomeroy and Wiebe [12], a wide variety of potential sources of substrates exist, among them the exudates from algae, which should be maximal during blooms. However, since actively growing phytoplankton usually release ≤10% of their photosynthate as dissolved products (e. g. [5]), bacteria could not be expected to process more than this amount unless there were algal cell lysis or cell breakage due to grazing. In the initial period of the spring blooms in Conception Bay, few zooplankters were present and their development time at 0° approaches 70 days [6]. There was no evidence of algal cells lysing. Thus there appears to be a time window at the start of the spring bloom when the algae are growing rapidly and accumulating, but most of their production is not available to the bacteria.

The third problem is one of data interpretation. Investigators studying bacterial activity in cold temperate spring blooms have arrived at different conclusions using apparently similar methods: direct bacterial counts as a measure of biomass and thymidine or leucine incorporation as a measure of bacterial activity. For example, van Boekel et al. [15] reported that the biomass of bacteria and protozoa remained low during a *Phaeocystis* bloom in the North Sea until the bloom started to decline. However, Rivkin et al. [14], while noting that the biomass of bacteria was low in a Conception Bay spring bloom, found rapid per-cell growth rates for the bacteria, comparable to those in temperate waters. Rivkin et al. [14] have compared their bacterial productivity data with those in Pomeroy et al. [13] and concluded that the discrepancy between the two data sets involves the conversion factors for uptake of radioactive thymidine to bacterial growth rates rather than in the data themselves.

Since the bacterial biomass in both studies [14,13] was equally low, the bacterial productivity data would not necessarily dispute the respiration results of Pomeroy and Deibel [10] or Pomeroy et al. [13]. Thymidine uptake is an extremely sensitive assay (nanomoles of uptake), while respiration measurements are orders of magnitude less sensitive (micromoles of consumption); whether the bacteria were slightly active or extremely active as measured by thymidine could not be resolved by respiration measurement. However, the pure culture data of Wiebe et al. [17] are not resolved since both the thymidine uptake technique [14] and microscopic cell counting over time [17] assess directly, albeit very differently, the question of the growth rates of bacteria. The discrepancy between the pure-culture growth rates and the thymidine incorporation rates need to be re-examined.

An explicit requirement of the hypothesis is that during the early stages of cold temperate algal blooms, substrate availability is minimal and thus the microbial loop is not operating significantly. The question of where substrates are generated has been addressed briefly above, but deserves some amplification, for it sheds light on how the microbial loop functions. In 1989 Jumars et al. published a paper entitled “Closing the microbial loop: dissolved carbon pathway to heterotrophic bacteria from incomplete ingestion, digestion and absorption in animals” in which they argue that zooplankton grazing is required to stimulate the microbial loop [2]. It is not algae per se that stimulate the microbial loop but rather their demise and release of cell constituents that provides the engine for microbial loop activity. We see our results in this context: an active microbial loop cannot be implemented under spring-bloom conditions until zooplankton grazing commences. In the case of cold-water spring blooms, zooplankton are scarce initially. The microbial loop activity must await their development. This may also be the reason that a number of investigators have found little relationship between algal abundance and bacterial abundance and activity. Time of sampling may be a major factor in producing seemingly conflicting conclusions.

This field is developing rapidly. While we believe some of the conflicts can be rationalized as discussed, there are several problems that need to be addressed. First, there is a need to document the source or sources of substrates for bacteria; while the grazing hypothesis is a tempting one, there is little direct proof that it is correct. Second, there appears to be a discrepancy between thymidine uptake and direct counting procedures to measure bacterial growth rates. Perhaps accounting for cell mass changes rather than cell numbers per se might resolve this issue. Or there may be a problem in relating the number of DNA replicons measured as thymidine uptake to cell numbers. Finally, physiological and biochemical studies are needed to define the mechanism(s) that are responsible for the observed elevated substrate requirement of bacteria growing at annual minimum temperatures. Nedwell and Rutter [4] have provided evidence for one possible mechanism, a decrease of specific affinity for substrate. Their results should be expanded to a wide selection of bacteria, and in addition other mechanisms, e. g. transport of substrate across the cell membrane, should be investigated.

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