Bacteria–Organic Matter Coupling and Its Significance for Oceanic Carbon Cycling

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Abstract. This paper synthesizes current ideas on the role of the microbial loop in carbon fluxes in the ocean and proposes some directions for future research. Organic matter flux into bacteria is highly variable, which can significantly influence the pathways of carbon flow in the ocean. A goal for future research is to elucidate the mechanistic bases of bacteria–organic matter coupling. This research should take into consideration the micrometer-scale distribution of bacteria and the composition, structure, and dynamics of the organic matter field in the bacterium's microhabitat. The ideas on the interactions of bacteria with the particulate organic phase need to be revised in view of recent findings of highly abundant, previously unknown particles ranging in size from nanometers to hundreds of micrometers. The "hot-spots" in the distribution of organic matter and remineralized nutrients can influence the rates as well as the direction of biogeochemical fluxes. Slow-to-degrade dissolved organic matter (DOM) may be produced because of loose bacteria–organic matter coupling resulting in DOM storage. Its use at a later time and place has profound implications for carbon fluxes and food web dynamics. A fundamental research need for the future is to understand the ecological interactions among the members of the microbial loop in an appropriate microhabitat context. While this goal was previously intractable, new molecular and optical techniques should make it possible to understand the biogeochemical activities of the microbial loop in terms of the ecology and evolution of pelagic microbial communities.

Introduction

Larry Pomeroy's seminal paper [49] revolutionized our concepts of the ocean's food web by proposing that microorganisms mediate a large fraction of the energy flow in pelagic marine ecosystems. Before 1974, bacteria and protozoa were not included as significant components of food web models (e.g., 61). Pomeroy argued forcefully that heterotrophic microorganisms, the "unseen strands in the ocean's food web," must be incorporated into ecosystem models. This bold departure from the then-entrenched concept of the grazing food chain has provided the agenda for marine microbiologists for the last two decades, namely the development of a

quantitative and mechanistic framework for incorporating microbial processes into the ocean's food web. Recent emphasis on global carbon cycling has further underscored the importance of this goal.

Quantitative studies that followed [26, 30, 33, 34, 42, 67] confirmed Pomeroy's conclusion by showing that major fluxes of carbon in the ocean's euphotic zone occur in the pathway: dissolved organic matter (DOM) → bacteria → protozoa → metazoa (Microbial Loop; [9]). The microbial loop is therefore a significant biological force in shaping the spatial and temporal patterns of the distribution of the bioelements C, N, P, and Fe, and there is a need to understand how to incorporate the microbial processes into models of the variability in the ocean's biogeochemical state.

During the last 10 years marine microbiologists had become complacent with the generalizations that the microbial loop transfers organic matter equivalent to about one-half of the local primary production from the dissolved phase into the particulate phase, efficiently remineralizes the organic matter entering it, and transfers some carbon to the higher trophic levels. However, recent work indicates that bacterially-mediated fluxes of bioelements are highly variable, both quantitatively and qualitatively. Carbon flux into bacteria can vary from 0 to > 100% of local primary production [51]. Further, depending on the nutrient status of their microenvironment, bacteria can switch between being net-consumers and net-producers of remineralized N and P [64]; this role variability has implications for C, N, and P flux pathways and for predictive ecosystem models.

The new challenge in the conceptual evolution of the microbial loop is to uncover the causes and mechanisms of this variability. We have come a long way since Pomeroy's initial discovery [49] in quantifying C, N, and P fluxes into bacteria and the microbial loop. Regulation of fluxes is the central problem in understanding the biogeochemical role of the microbial loop, but it is a complex and challenging problem. This is because fluxes into bacteria are derived from varied pools of organic and inorganic materials which are distributed heterogeneously in space and time and are further influenced by biochemical, trophic, and physicochemical interactions in the environment. The first step in solving this problem is to develop a suitable conceptual framework for addressing the regulation of material fluxes through bacteria, and this is our goal in writing this paper.

We suggest thinking of the problem from the standpoint of the bacterium and how it interacts metabolically with the materials and microbes in its microenvironment to achieve the fluxes and biochemical transformations of biogeochemical significance. Such an integrative approach will require the development of new types of methods, including optical and molecular methods.

We first discuss the small-scale distribution and dynamics of organic matter in the environment of bacteria and its relationship to bacterial activity, and then address the problem of regulation of carbon flux into bacteria and its biogeochemical implications.

Particulate and Dissolved Phases in the Environment of Bacteria

There has been much interest in distinguishing between the metabolic activities of particle-attached and free bacteria [6, 14], and it is thought that attached bacteria

inhabit organically rich microzones while free bacteria live in low-nutrient environments. Particles create spatial heterogeneity in the distribution of organic matter, remineralized nutrients, and the abundance and species composition of microorganisms. The marine particulate phase harbors rich bacterial populations on the order of 10^8–10^9 ml^{-1} (three orders of magnitude greater than in the surrounding waters [2]). DeLong et al. [21] found bacterial populations on marine snow enriched with respect to particle specialists relative to the surrounding water. These specialists may have biochemical adaptations (e.g., high activities of hydrolytic enzymes; [39, 60] for rapidly hydrolyzing the particulate organic matter (POM). Because of loose hydrolysis-uptake coupling [60] particle-bound bacteria can render the particle a source of DOM, thus creating heterogeneity in DOM distribution. Aggregates such as marine snow can contain very high concentrations of nutrients [56], rendering it and the surrounding microenvironment a "hot spot" of remineralized nutrients. Further, the interior of large particles can become anaerobic [1] and support denitrification [37] and sulfate reduction [57]. These are biogeochemical transformations not predicted by models that assume random distribution of organic matter and microbes in the pelagic environment.

The above discussion shows that there is support for the idea, in general terms, that organic particles in seawater create heterogeneity in the distribution of nutrients and microbes in the microenvironment and thereby influence small-scale spatial variability in the rate and type of bacterial activities of biogeochemical significance. This idea is by no means new and has been intensively studied and passionately debated for decades in the context of importance of particles in the pelagic ocean as microzones for bacterial metabolism and growth [6, 14, 15, 17, 23, 36, 38, 43, 65], yet our understanding of POM–bacteria interactions is quite limited. This may be because the traditional distinction between the particulate and the dissolved phases is arbitrary and operational (e.g., filterability) and not based on the nature or the structure of the organic matter field in situ or how bacteria "see" it. Conceptual models have drawn rigid boundaries around the particles to separate them from the dissolved phase. While convenient, such demarcation is probably not experienced by bacteria in seawater where DOM and POM "blend" to create a dynamic continuum of organic matter with which bacteria interact. A fundamental difficulty in understanding bacteria–organic matter interaction is that we do not know the structure and composition of the organic matter field, which includes other microorganisms, in the bacterium's microenvironment. Marine microbiologists have recognized this for a long time, but the problem has been considered intractable. Recent discoveries (below) on the physical nature of DOM and POM pools now provide cause for optimism that we may yet gain insights into the fine structure of the organic matter field of bacteria.

A number of "new" types of particles have recently been discovered at abundances that are orders of magnitude greater than the "classical" particles, and this changes our perception of the structure of the organic matter field at the micrometer scale. Koike et al. [45] discovered that surface seawater contains 0.3 to 1.3-μm-sized organic particles on the order of 10^7 ml^{-1} not previously observed by light microscopy. Alldredge et al. [3] discovered that surface seawater contains particles (10^2–10^4 ml^{-1}) that stain with the acid mucopolysaccharide stain alcian blue (transparent exopolymer particles, TEP). TEP are highly variable in size (3 to > 100 μm) and their forms include sheets, strings, and bundles of filaments.

Importantly for our discussion of bacteria–organic matter coupling, TEP is often colonized by bacteria (28–68% of the bacteria were found attached to TEP [3]). Wells and Goldberg [63] discovered highly abundant (10^7–10^{10} ml^{-1} colloidal size (<120 nm) particles in seawater. The pools of all these "new" particles are highly dynamic and the origins and degradation of these particles are believed to be dominantly biological.

Much remains to be learned about the chemical composition, origins, fates, and the ecological and biogeochemical roles of these "new" particles. However, their sheer abundance and the association of bacteria, at least with some of these particles, shows that traditional views of the interactions of bacteria with the particulate phase should be revised. One might envision that these "new" particles form a gel-like organic matrix within which the "classical" particles are embedded, and this matrix forms the physical context within which the organisms of the microbial loop operate, interact, and effect material fluxes. In order to build a unified picture of the organic matter field we suggest treating the living organisms of the microbial loop as part of this organic matter field. Further, the DOM pool should be considered along with the particulate phase so as to treat the organic matter pool as a continuum and to accommodate the POM ⟷ DOM transitions that occur in seawater.

Dissolved polymers constitute a substantial fraction of the organic matter pool and apparently play a pivotal role in bacteria–organic matter coupling [18]. Peptides and polysaccharides are quantitatively important potential sources of bacterial nutrients and are 1–2 orders of magnitude more abundant than the monomeric direct substrates produced by their hydrolyses. Polysaccharides account for ~50% of the DOC in surface waters [13]. The majority of organic matter flux from particulate sources into bacteria probably passes through a polymer pool in the pathway: particulate source → polymers → direct substrate → bacteria. Therefore, it is important to determine the small-scale distribution and dynamics of polymeric substrates, how they are produced from and interact with the particulate phase in the bacterium's environment, and how their conversion to direct substrates regulates the flux of C, N, and P into bacteria.

In sum, controls on carbon flux into bacteria are multifaceted, reflecting the composition and dynamics of the organic and inorganic matter in the bacterium's microenvironment. Material fluxes are also controlled by factors that regulate metabolic rates, population size, and species composition of bacteria. Since multiple factors may simultaneously contribute to the control of carbon flux, an integrative view is used in the next section where we discuss ideas on bacteria–organic matter coupling and the biogeochemical consequences of its variability.

Bacteria–Organic Matter Coupling

Conceptually, bacteria–phytoplankton coupling for carbon flux (F_c) implies a measure of the fraction of primary production consumed by the co-occurring bacteria community. Operationally, however, F_c is determined as the ratio of contemporaneously measured bacterial carbon demand (BCD) and primary production (PP); F_c = BCD/PP. The use of colocalized, concurrent measurements of bacteria and phytoplankton production can be misleading and obscure the nature of their trophic

relationship. For example, any environment, no matter what the F_c during the day, will be considered hyper-coupled ($F_c > 1$) during the night because photosynthesis has ceased. Such oscillations in F_c will result not only from diel, but also diurnal or seasonal cycles. What then is the relevant time frame for averaging PP and BCD to decide whether a system is coupled or uncoupled? Another problem is that the concept of coupling implies an intimacy between the primary and bacterial secondary productivity, which may not exist. There may be situations where none of the primary production is consumed by the resident bacteria, but BCD equals PP due to consumption of preformed organic (allocthonous) organic matter. Clearly, the concept of coupling is not commensurate with the operationally defined F_c. This is not to imply that F_c is not a useful measure. On the contrary, this ratio provides an indication of whether, at a given place and within a proscribed time, an ecosystem can be considered net-heterotrophic or net-autotrophic. This is important for determining the potential for and patterns of CO_2 transfer between the ocean and atmosphere. We suggest that the term "coupling" be reserved for those situations where the sources and sinks in the transfer are known or explicitly hypothesized.

The most celebrated case of uncoupling ($F_c = \sim 0$) was discovered by Pomeroy and others [50, 51] during early spring in Newfoundland coastal waters. They found that despite a phytoplankton bloom, BCD remained unmeasurable for weeks, apparently because the near-zero water temperature inhibited bacterial metabolism but not phytoplankton production. Because of low temperature, bacteria could not use phytoplankton carbon. Importantly, the low temperature inhibition of BCD could be alleviated by high substrate concentrations which was consistent with a higher BCD at the chlorophyll a maximum. We infer from these observations that rapid, cell-specific BCD may also have occurred outside the chlorophyll a maximum but was restricted to microscale substrate "hot spots" (e.g., on or near particles). However, the abundance of "hot-spots" may have been too low to cause high F_c. As the abundance of hot-spots and bacteria in them increases (e.g., phytoplankton senescence or heavy exudation; [5, 16, 51]) BCD would increase even at low temperature.

Is there evidence that pelagic bacteria are capable of bursts of rapid growth, and therefore enhanced carbon flux, in enriched (micro)environments? Jöborn, Cochlan, Steward, Smith, Rico-Mora, Azam (unpublished) enriched seawater with freeze/thaw killed cells of *Nitzschia angularis*. Bacterial microcolonies appeared on diatom cells within 8 h, apparently by growth in situ. Martinez and Azam [47] grew pelagic bacteria in pure cultures in enriched media and found doubling times as low as 13.2 min (isolate S8, apparently *Vibrio splendidus*, isolated off Scripps Pier). This isolate became dominant, based on DNA/DNA hybridization experiments, during a seasonal study [54] and therefore might be considered adapted to growth in seawater. Smith and Azam (unpublished) found rapid growth of bacteria on laboratory-produced fresh phytoaggregates (bacterial doubling times of 2–3 h). Thus, at least some pelagic bacteria have the potential for much faster growth than the assemblage average, and are capable of "bursts" of growth. The maintenance of rapid growth potential suggests the hypothesis that pelagic bacteria are adapted to taking advantage of high substrate concentrations in nutrient hot spots. This hypothesis is also consistent with the simultaneous presence of low-flow and high-flow D-glucose transport systems in marine bacteria [7].

Not all enriched microzones support rapid growth, and research on bacterial utilization of organic matter on marine aggregates has lead to some surprising

results. Field-collected marine snow harbors very high populations (10^8–10^9 ml^{-1}) of slowly growing bacteria (generally, doubling times of 0.5–10 days; [2]). Particles collected in sediment traps have been considered inhospitable sites for bacterial growth [38]. Presumably, the "culture conditions" in the aggregates' microenvironment deteriorate and inhibit growth despite abundant residual nutrients, a situation reminiscent of stationary cultures of bacteria [12]. In the context of bacteria–organic matter coupling it is interesting that the aggregates experience intense activity of hydrolytic enzymes (protease, glucosidase, phosphatase, and chitinase) which solubilize POM. However, due to low BCD most hydrolysate diffuses away resulting in loose coupling in the flux of the aggregate's carbon to the attached bacteria [60]. The uncoupling may occur because the hydrolysates produced by various enzymes react to produce slow-to-degrade condensation products [60]. This could be important for DOM storage which is discussed below.

Metabolic Accessibility of Organic Matter

Bacteria–organic matter coupling depends on the utilizability of organic matter by the resident bacterial assemblage. Historically, it has been thought that a small, fast-turnover pool of DOM supports most of the carbon flux into bacteria, while 90–98% of the total DOM is old and nonutilizable (but see [44]). However, in recent years, the distinction between utilizable and nonutilizable DOM has become fuzzy because of indications of active exchange between the two pools. Keil and Kirchman [40] observed that peptides added to seawater become slow-to-degrade within hours to days, possibly because of condensation with carbohydrates. This is consistent with previous findings that only a fraction of amino acids and peptides in seawater is available for bacterial uptake [19, 40], particularly at depth [11]. It would be interesting to determine whether some DOM components other than peptides and carbohydrates also become slow-to-degrade.

The transformation of utilizable DOM into slow-to-degrade DOM introduces a temporal element in DOM utilizability. If bacterial uptake of utilizable DOM is slowed (by factors unrelated to DOM composition) the utilizable DOM will have more time to undergo reactions including those rendering it slow-to-degrade. Consequently, utilization of newly produced utilizable DOM is a race against time for bacteria. On the other hand, some newly produced nonutilizable DOM may become utilizable with time (e.g., by UV radiation; [48]).

In view of the race against time, it may be advantageous for bacteria to take up new DOM in tight spatial and temporal coupling with phytoplankton and particles. Bacteria may interact with diffusible and surface-bound algal exudates by chemokinesis [16] and use ectohydrolases to hydrolyze surface mucus of phytoplankton [8]. Bacteria clustering around phytoplankton would be exposed to high concentrations of "fresh" DOM. Similar considerations should apply to bacteria–particle interactions. Simultaneous attack of several bacterial ectohydrolases on the particle would create high concentrations of dissolved compounds in its microenvironment. Because of their high concentrations, the hydrolysates may react more rapidly within the particle than after diffusion into the bulk-phase water. Chemical reactions could change the utilizability of the hydrolysates, and the reaction products may include slow-to-degrade DOM [60]. For instance, newly produced peptides

and carbohydrates may undergo glucosylation [40]. Further, some of the hydrolysis products may already be slow-to-degrade. The slow-to-degrade DOM may diffuse faster than it can be used by attached bacteria and become "stored" in the DOM pool.

Enzymatic attack can also change the utilizability and nutritive value of POM by differentially removing specific components. Smith et al. [60] found much higher protease than glucosidase activity on marine snow and suggested that protein is solubilized more efficiently than polysaccharide. This could render the residual POM less suitable for bacterial growth. As an indirect effect of hydrolyses, metals bound to organic moeities in the POM could be released and this could inhibit the utilization of otherwise rapidly utilizable compounds. For instance, Cu and Cd are present at very high concentrations in marine snow [31], and their mobilization by enzyme attack could inhibit DOM utilization [60].

DOM Storage

DOM could accumulate if mechanisms to produce it were operating but bacteria could not utilize it rapidly enough, because it became slow-to-degrade or because bacterial growth was inhibited by factors unrelated to substrate availability. The stored DOM, persisting for weeks to months, will be subject to downward transport through mixing [55, 62]. Weak bacteria–organic matter coupling could therefore increase the downward flux not only of POM [51] but also DOM. The "stored" DOM could be utilized later and possibly after export to a different location.

Diel variability in bacteria–phytoplankton coupling is an obvious example of such temporal export of DOM. The use of DOM at night causes hyper-coupling because bacterial metabolism continues in the absence of contemporaneous local primary production [27]. Long-term (e.g., seasonal) alternation between poor coupling and hyper-coupling can have important biogeochemical consequences. Environments that experience intense phytoplankton blooms followed by periods of low primary productivity (e.g., North Arabian Sea) may exhibit low F_c during the bloom followed by $F_c >> 1$ during the oligotrophic period if significant organic matter is stored during the bloom. Such variation in F_c would render the oligotrophic period net-heterotrophic, and this has implications for carbon cycling and air–sea exchange of CO_2. Ducklow [22] found F_c in the north Arabian Sea (integrated from 100–1000 m) was an order of magnitude greater than the depth-dissipation of sinking flux. These data are consistent with the hypothesis that poor coupling during the southwest monsoon results in slow-to-degrade DOM, and the vertical transport of DOM (rather than sinking POM) supports the majority of mesopelagic bacterial carbon demand.

Temporal export of DOM may be important in the flows of materials and energy in Antarctic waters. The productive photic period may generate slow-to-degrade DOM which could support bacterial production during winter and supply energy to higher trophic levels [10]. In the case of Antarctic winter it is interesting to speculate that, in addition to the slow bacteria production [35], turbulence-induced conversion of DOM to POM could be significant in mediating DOM → POM transition. We wonder if turbulence created by krill swarms might mediate significant DOM → POM transition thus making DOM available to krill while bypassing

the microbial loop. Turbulence-induced particle formation could also stimulate bacterial utilization of DOM [41].

Oceanic DOM is one of the largest carbon pools on Earth. Even though this pool is in contact with abundant and metabolically diverse populations of bacteria it turns over extremely slowly, the average age of DOC being on the order of a few hundred to a few thousand years. Bacteria–DOM interaction appears to defy the principle of microbial infallibility ("It is probably not unscientific to suggest that somewhere or other some organism exists which can, under suitable conditions, oxidize any substance which is theoretically capable of being oxidized"; [28]). While the DOM pool does turnover slowly, why is it that no bacterium has arisen to rapidly exploit it? Perhaps there are such insurmountable chemical constraints that bacteria have not managed to evolve effective enzymes to metabolize the old DOM? It may be that the diversity of molecular species produced in seawater is so great that bacteria would need an unrealistically diverse enzyme repertoire to metabolize them. Is the molecular diversity too great even for multispecies assemblages to evolve the necessary metabolic enzymes? Perhaps the chemical structures of the DOM pool are constantly being modified by biochemical and physicochemical forces in the sea in such varied and "unpredictable" ways (e.g., by the combined actions of hydrolases and condensation reactions) that it is not adaptive for bacteria to keep up with the changing biochemical challenges.

Trophic Interactions Influencing Carbon Flux into Bacteria

Bacterial abundance generally remains within narrow limits even during phytoplankton blooms (e.g., [24, 46]), presumably due to predatory pressure by protozoa [66] and viruses [32, 53], and the control of bacterial population size limits F_b. We consider the following scenario: During a phytoplankton bloom potentially-utilizable DOM and POM are rapidly produced but remain largely unutilized despite rapid bacterial growth because predation keeps the bacterial population in check. The resulting poor coupling then is not due to inhibition of bacterial growth nor restriction of carbon input from phytoplankton but control of population size. This scenario (as distinct from other causes of uncoupling discussed earlier, e.g., [51]) is probably common during the course of phytoplankton blooms and may result in the accumulation of DOM and POM.

Protozoa and bacteria compete for a common resource, namely fine POM, which bacteria use by solubilizing via ectohydrolases and protozoa by direct ingestion. Protozoa can ingest 0.1- to 1.0-μm particles [58], colloids [59], and viruses [29], a size range of organic matter that is a potential substrate for bacteria. Carbon flux into bacteria could therefore be restricted if protozoa rapidly removed fine POM. On the other hand, protozoa feeding on fine detritus may release DOM that could be available to bacteria [4]. We suggest, therefore, that protozoa and bacteria should be modeled as competitors for fine detritus, possibly including the "new" particles discussed above.

Viral infection can also control bacteria populations [52] and this could reduce F_c. However, DOM produced by virus-induced bacteria lysis [52] may be recycled and result in increased carbon flux into bacteria and higher F_c [25].

It thus appears that control of carbon flow into bacteria depends on interactions between phytoplankton, bacteria, viruses, and protozoa [5]. These should be stud-

ied in terms of the activities of whole microbial consortia, and for this purpose phytoplankton belong in the microbial loop. Phytoplankton have historically been the domain of the grazer food chain (not surprising since they are at the base of the grazer food chain), but they should also be considered "full members" of the microbial loop. Phytoplankton are (obviously) microorganisms and they interact with other members of the microbial loop at a similar space scale [5]. Some phytoplankton are prokaryotes (*Synechococcus* and *prochlorophytes*) while some others are protozoa (mixotrophs). If the study of the microbial food web had preceded the grazing food chain then surely phytoplankton, along with other microorganisms, would be in the microbial food web (while at the same time being important for supplying material and energy for the "grazer loop"). Indeed, it is futile to separate the microbial loop and the grazing food chain along disciplinary lines. For instance, bacteria–phytoplankton coupling may be profoundly influenced by zooplankton grazing activity.

Control of Carbon Flow by Inorganic Nutrients

In the traditional view, bacteria mineralize organic matter and release N and P for the primary producers, but it now appears that bacterial growth can at times be N or P limited and bacteria can become competitors of phytoplankton for N and P [8, 64, 68]. Partitioning of inorganic nutrients between bacteria and phytoplankton can therefore control carbon flux into bacteria as well as the rate of primary production (which in turn controls carbon flux into bacteria). We have previously developed the argument that whether bacteria are net producers or net consumers of inorganic nutrients depends on the nutritional status of the bacterium's microenvironment [8]. Bacteria are net remineralizers in energy-rich microenvironments with low C/N and C/P (near a phytoplankton cell or on a decomposing particle?). Bacteria in the "minimum medium" (e.g., bulk phase seawater) are likely net consumers of nutrients. Referring back to the discussion of the organic matter field in the bacterium's microenvironment, we can now see how inorganic nutrient fluxes might be "structured" by the distribution patterns of materials and microbes at the micrometer scale. Particulate loci of suitable biochemical composition would be "hot spots" of remineralized nutrient production. Protozoa would release pulses of remineralized nutrients (possibly including reduced iron). Such production loci would expose bacteria and phytoplankton to heterogeneous and time-varying concentrations of nutrients. The small-scale spatial organization of the organic matter field and microbial consortia should thus be considered for understanding the mechanisms of C, N, and P fluxes.

Future Directions

We have attempted to develop a conceptual context for addressing questions of bacteria–organic matter coupling. The microscale structure of the organic matter field and its dynamics expose bacteria to gradients of organic and inorganic nutrients that can profoundly influence bacteria–organic matter coupling and C, N, and P fluxes. A challenge for the future is to incorporate the microhabitat structure, the new "strands in the ocean's food web," into the concept of the microbial loop.

New approaches will be needed for this phase of studying the role of the microbial loop. Some techniques are already on the horizon. For instance, an optical method has recently been developed (Krembs, Juhl, Strickler, submitted) that has the potential to uncover the microscale distribution of organisms and particles. Coupling of such optical methods with techniques for identifying and measuring the activities of individual microbial cells is feasible in principle. Individual cell identification is now possible with the use of fluorescently tagged species-specific oligonucleotide probes [20]. Single-cell growth, metabolic activity, and ectohydrolase measurements should be possible by techniques such as microautoradiography and flow-cytometry coupled with the use of radiolabeled or fluorogenic substrates.

In conjunction with activity measurements it is important to determine the chemical composition of the organic matter. We marine microbiologists often accuse the chemists of ignoring microbial processes, but frequently we are ourselves guilty of ignoring the chemical environment within which the microbial processes take place. Analyzing organic matter in seawater is a formidable challenge, and consequently the focus has been on selected components, notably amino acids, for which methods of analysis have been available. We need to develop aggressively the means to determine the composition of the organic matter in the microbial environments. This effort should include the analysis of the particulate phase as well which has so far received less attention.

Ectoenzymology of marine bacteria has emerged as a major theme in bacteria–organic matter coupling during the last 10 years [18]. Hydrolytic ectoenzymes may play a pivotal role in mediating and regulating organic matter fluxes from the polymeric and particulate phases into the pool of direct substrates. As such, these enzymes could be the biochemical conduit that channels organic matter from substrates to the transmembrane transport systems of bacteria. The cellular regulation of the enzyme activities can therefore influence bacteria–organic matter coupling. Further, the tightness of coupling between the hydrolysis and the uptake of the hydrolysis products is important in determining the pathway followed by the hydrolysate. In order to understand the mechanistic bases of bacteria–organic matter coupling. The regulation of enzyme expression in marine bacteria needs to be examined at the molecular level, yet in an appropriate ecosystem context.

Finally, a fundamental challenge for the future is to understand the ecological interactions among the members of the microbial loop, because such interactions form the bases of the biogeochemical fluxes and because such understanding is critical to progress in marine microbial ecology. The study of microbial ecology has until recently been pursued either by visionaries or fools. The exceedingly small size of the organisms and their environments has, until recently, made it extremely challenging to address basic questions such as: what organisms constitute natural microbial assemblages, what are *their* environments like, what are the in situ metabolic activities and rates, what is the community structure and dynamics, and what adaptations for survival are used by the organisms? The advent of molecular biology has, for the first time, made it possible, at least in principle, to address these previously intractable issues. Their resolution promises to be exciting and should make it possible to understand the biogeochemical activities of the microbial loop in terms of the ecology and evolution of the pelagic microbial communities.

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