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Biogeosciences
Discussions

Interactive comment on "High growth potential and activity of 0.2 µm filterable bacteria habitually present in coastal seawater" *by* Yumiko Obayashi and Satoru Suzuki

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Thank you very much for the helpful comments. Here is our reply to each comment.

—Comment— The article of Obayashi and Suzuki describes two long experiments (at different temperatures) focusing on the growth of marine coastal 0.2 μm filterable bacteria in the absence of grazers (but in the presence of viruses!). As the authors state, the experimental setup was designed to study the lifetime of the dissolved extracellular hydrolytic enzyme activities in seawater. Due to this change of plan, I do not find the experimental design being optimal to address the high growth potential of 0.2 μm filterable coastal bacteria. The volume is too small given the long incubation time (19 days). It is not clear how at T0 the authors can estimate the <0.2 μm bacterial abundance if all

or most of the cells escape in the filtrate if they are using 0.2 μm polycarbonate filters (there is no mention in the text, that they have used 0.02 μm alumina oxide filters to solve this issue or alternatively flow cytometry). Measurements of bacterial volume or bacterial size would have been important to show.

—Reply—

As the Referee pointed out, our experiments might not be optimized way to show the growth of 0.2 μm filterable bacteria directly, however, the results of our experiments which demonstrated rapid increase of the number of prokaryotes trapped on 0.2 μm filter in the FSW bottle (originally 0.2 μm filtered seawater) also show the evidence of high growth potential of filterable bacteria in seawater.

We used 1-L polycarbonate bottles for the experiments because of the size limitation of the incubators (to incubate many bottles in the same condition). The actual starting volume of the water in each bottle was about 1.2 L, and more than half of the water still remained at the end of the experiments.

In this study, we did not estimate abundances of the "actual <0.2 μm" cells and viruses in the sample during the experiments, so that we were not able to mention "obligate ultramicrobacteria". In the experiments, the number of prokaryotes was counted on 0.2 μm filter after filtration (usually 2 mL) and the results demonstrated rapid increase of the number of the >0.2 μm cells in the FSW bottles. For Day 0 sample of FSW, 3 mL of the water sample was filtered onto black polycarbonate 0.2 μm filter after staining with DAPI. More than 200 fields were observed under fluorescent microscope (x1000) and very few cells were found on the filter. Although the estimated cell numbers on Day 0 in FSW could include much uncertainties, it was clear that the number of cells on Day 0 in FSW was much lower (almost 2 orders of magnitude lower) than those on later days of FSW bottles and those in UNF bottles.

All bacteria we counted here were larger than 0.2 μm.

—Comment— Within the microbial community, fundamental players (that were present in the experimental water, since water has been filtered onto 0.2 μm) like viruses and their role in shaping the microbial community have not been considered (no viral abundance data, no discussion on their role). I feel that this manuscript for "non-expert readers" will offer partial and incorrect information on the microbial dynamics that regulate the growth on marine bacteria.

—Reply—

As the Referee mentioned, viruses are also fundamental players in the microbial community. In our experiment, virus should be abundant both in FSW bottles and UNF bottles. We supposed that the viruses were similarly abundant in the both bottles at the beginning of the experiments and that the differences of the microbial community between in FSW and in UNF at the beginning of the experiments were the abundances of grazers and prokaryotes; no grazers and much lower abundance of prokaryotes in FSW bottles compared to UNF bottles. It can be supposed that the increasing of the number of prokaryotes in FSW bottles during the early stage of the experiments were attributed to no grazers and low competition condition for prokaryotes. The “seeds” of the increasing prokaryotes in FSW bottles should be filterable bacteria present in the FSW (0.2 μm filtrate) at the beginning of the experiments. On the other hand, decreasing of the number of prokaryotes during the later stage of the experiments should be due to the effect of viruses. We would like to add mentions of viruses and their roles in discussion part of the revised version of our manuscript.

—Comment— The references are not updated. If the field of ultra-micro bacteria is not popular in these days, grazing and viral lysis are still very hot-topics.

—Reply—

Thank you for the helpful comment about the references. We will update references especially for those about microbial dynamics in aquatic environments in the revised manuscript.

—Comment— Furthermore, given the next-generation sequencing technologies (NGS), it is necessary to compare the DGGE results (and interpretation) with NGS, also if it is challenging.

—Reply—

As the Referee said, NGS analysis is powerful tool to see all members in sample, whereas DGGE can selectively show abundant members. This is a rather merit of DGGE. The information from DGGE profiles combination with sequencing of the bands could provide visualized information about the abundant bacterial community (>0.2 μm) reconstructed in FSW bottles from the “seeds” in 0.2 μm filtrates. The results of DGGE do not represent whole structure of bacterial community, however, each bacterium which was detected in the DGGE was rightly present in the sample. From the results that typical marine bacteria (Alphaproteobacteria, Gammaproteobacteria, and Flavobacteria) were detected from FSW microcosms, we can say that the “seeds” of these bacteria were existed in 0.2 μm filtrates at the beginning of the experiments, indicating that these bacteria with filterable form (small or flexible enough to pass through 0.2 μm filter) were present in the original coastal seawater.

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