

Responses to anonymous referee 2

Dear editor and reviewers,

The authors would like to greatly thank the anonymous referee for his/her interest of our work. You will find below our responses to his/her valuable comments.

Specific comments:

Abstract, chapter 3.2, Tab 1+2: Did you mention the right number of isolates? Abstract 52, chapter 3.2: 54 minus the one strain not recovered = 53, 54 in Tab 1 and 53 in Tab 2.

The exact number was 54 and we modified the text accordingly.

Page 4423 line 7-9: Cell counting of AAP bacteria by the IREM method need to be interpreted with caution (Schwalbach and Fuhrman, 2005). That is the reason why abundance and significance of AAP bacteria were overestimated in the studies from Kolber et al. (2000, 2001). Data of Schwalbach and Fuhrman (2005) raise the question about where AA Photosynthesis is advantageous in marine ecosystems.

We modified the references and added that of Schwalbach and Fuhrman (2005).

Page 4426 line 1: In which depths did the DCM occur? Give values or at least refer to section 3.2.

We referred to Table 1 where DCM depths are indicated according to the samples used.

Page 4426 line 25: Illumination by which device? Give name, manufacturer, etc. of the used lamp for reproducibility regarding the spectrum of wavelengths.

We added the requested information.

Page 4426 line 28: Did you screen all plates and all media, respectively, for AAP bacteria? Refer media (MAD, MiA, MA?).

This information was given in the first sentence of chapter 3.2. However, we modified the following sentence to clarify (see below).

“Briefly, after up to 7-8 weeks of incubation at 18°C, colonies grown on the MA, MiA and MAD media were illuminated by a set of Nichia blue light emitting diodes (470 nm). Infra-red fluorescence from BChl *a*-containing colonies was registered by a CCD camera (Photon Systems Instruments Ltd., Brno, Czechia) protected by a long pass >850 nm glass filter (Oriel 51360, USA).”

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Page 4428 section 2.4: Not clear from which samples you generated RT pufM libraries.

This has been clarified (see our answer to referee 1).

Pages 4428 line 21: Why did you choose the nucleotide based primer pufMF of Béjà et al. (2002) for cDNA amplification of your diversity study? In 2005 Yutin and colleagues published fully degenerate pufM primers recovering a wider diversity of marine AANPs than the nucleotide-based original pufM primers from Achenbach et al. (2001) and Béjà et al. (2002) and were used for e.g diversity analyses by DGGE (Yutin et al. 2008). Also

other studies, e.g. Hu et al. (2006), used pufMF as forward primer and reported of abundant presence of AAP clones affiliated to the Gammaproteobacteria in oxic oceanic surface waters. Furthermore, primer pufMF discriminates several Roseobacter clones and strains. Maybe the choice of a more degenerated forward primer would have revealed another diversity pattern of the pufM transcripts and weaken the strong gap between culture-dependent and culture-independent methods.

In the sentence introducing the primers, we argue that we selected them primers based on their higher specificity and efficiency (yield of PCR products). We added the reference Lehours *et al.* (2010) where the same primers were used after testing several pairs of primers. We do not think that these primers could explain the gaps between culture-dependent and culture-independent methods. When used in samples from other marine systems (coastal environment in the English Channel and in the Arctic ocean), the same primer pair did not show such an overdominance of *Gammaproteobacteria* and *Roseobacter*-like clones were also abundantly recovered.

Tab 3: Add a column with the accession numbers of the representative clones or refer it in the caption. May add a column of the phylogenetic affiliation or give abbreviations in upper case.

The columns have been added.

Fig 1: What is the meaning of “SeaWiFS”? What is the meaning of “both”? Delete it and replace by e.g. additionally, etc.

SeaWifs stands for Sea-viewing Wide Field-of-view Sensor, a very well know NASA mission. “Both” has been deleted and “additionally” has been added to the sentence.

Technical comments:

Abstract page 4422 line 18: : : :affiliated to: : :

Pages 4431 line 1-2: Syntax : : : from 21.4_C (station 5) to 26.9_C in the western basin.

Page 4432 line 7: : : :synthesize: : :

Page 4436 line 22: dimethylsulphoniopropionate

Page 4437 line 16: BChl-a synthesis

Page 4438 line 29: : : :abundance. Moreover,: : :

Tab 2: Roseovarius halotolerans

Fig 2: Description of the phylogenetic groups is different. Choose second one: Alpha-4 Proteobacteria, Alpha-1-Proteobacteria

Fig 2 caption: Refer that Fig 2 deals with the sequences of the isolated AAP bacteria.

All technical comments have been taken into account.