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Biogeosciences Discussions

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Interactive Comment

Interactive comment on "Role of environmental factors for the vertical distribution (0–1000 m) of marine bacterial communities in the NW Mediterranean Sea" *by* J. F. Ghiglione et al.

J. F. Ghiglione et al.

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Answer to U. Stingl (Referee)

Answers to the referee are reported point by point. Changes in the text are located by the number of the corresponding line in the original manuscript:

Answer to Specific comments:

Specific comment 1: Papers proposed by the referee will be added in the revised version of the manuscript. See references above when answering specific comments.

Specific comment 2: Different aspects are discussed by the referee about (i) CE-SSCP compared to other fingerprinting techniques and (ii) the use of clone libraries from dif-

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ferent depths to unravel the physico-chemical and biological driving forces of bacterial community changes in the water column. (i) CE-SSCP compared to other fingerprinting techniques: Sensitivity: Recently several authors (Moeseneder et al., 1999 , Horz et al., 2001 , Casamayor et al., 2002 , Nunan et al., 2005 , Hong et al. 2007 and Smalla et al. 2007) compared the potential of different fingerprinting methods to unravel the diversity of bacterial communities from complex environmental samples. Although the fragments amplified comprised different variable regions and lengths, the structure of the microbial assemblages was generally the same, although some methods seem to perform less well in some specific situations. For example, Smalla et al. (2007) provided similar results on the bacterial community composition of four soils analysed by three fingerprinting methods, but T-RFLP patterns seemed to be less complex than DGGE and SSCP (on slab gels) patterns. Optimization of T-RFLP analysis by Moeseneder et al. (1993) showed that similar marine bacterial community assemblages were obtained with both DGGE and T-RFLP techniques, although "the T-RFLP approach and CE detection of OTUs was more sensitive, as indicated by the higher number of OTUs detected". Recently, the same result was obtained by comparing CE-SSCP with DGGE by Hong et al. 2007, who concluded that "CE-SSCP resolution was higher in terms of number of peaks resolved, reduced co-migration of distinct DNA sequences, and length and legibility of the DNA sequencing data of clones used to identify peaks." In a recent paper, we also show that CE-SSCP does not miss any major known groups, since up to 80% of the community peaks matched those obtained from a clone library of the same sample, accounting for 86.7% of the total fingerprinting area. Also, this paper shows that CE-SSCP is a reasonably quantitative measure of the relative abundance of the only taxon we could independently measure (Prochlorococcus, by flow cytometry). In the present paper, we do not claim that "CE-SSCP is the best profiling method". We are aware that each fingerprinting technique has its own advantages and inconveniences. The choice of the CE-SSCP technique has been based on the consideration put forth by Hong et al. (2007) and also by taking into account the number of samples to be analysed.

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Number of samples analysed: We agree with the reviewer that choosing a fingerprinting method depends on the question to be answer. A critical aspect in exploring the influence of environmental parameters as potential driving forces for shifts in bacterial community structure is the number of samples that can be analysed using a given technique. We agree that DGGE can have some advantage by giving the possibility to sequence the bands of interest. However, this technique cannot run as much as samples as we analysed, because of the calibration of the linear gradient of DNA denaturants that makes gel-to-gel comparison difficult and a maximum of 16-18 samples/gel. This problem has made the DGGE technique impractical for the fine comparison of large number of samples (Moeseneder et al. 1999). CE-SSCP, as T-RFLP, benefit from the recent application of capillary electrophoresis, which allows the reliable comparison of a theoretically infinite number of samples due to the inclusion of an internal standard running with each sample. As depicted in our Materials and Methods section, we have used the recent improvements in SSCP technique by the use of capillary electrophoresis (CE) and fluorescence detection (using a labeled primer) to compare the bacterial community structure of our samples. Similar methodology (polymer concentration, temperature, etc.) has been used by many other authors using the Applied Biosystem 310 automatic DNA analyzer for CE-SSCP analysis (Agogué et al. 2004 ; Delbès et al. 2000 , 2001; Duthoit et al. 2003 ; Ghiglione et al., 2005, 2007; Lee et al., 1996; Zumstein et al. 2000). Detection, sensibility and reproducibility of methods used in this study has been described on a previous paper (Ghiglione et al., 2005).

The co-migration problem when linking clone libraries with fingerprinting techniques: how many species are under a peak (or band)? Molecular fingerprinting techniques in general are restricted in giving information about richness, since they results in 30 to 40 peaks (or bands) in marine waters, which is far less than the real number of bacterial species living in the ocean. This is due to co-migration of PCR-products from different species within the same peak (or band), as depicted by several authors (Kowalchuk et al,. 1997; Vallaeys et al., 1997; Wintzingerode et al., 1997 ; Piceno et al., 1999; Casamayor et al., 2000; Schmalenberger and Tebbe, 2003). For instance, when using

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a fingerprinting technique, the identification of a particular peak (or band) on the basis of database information is not definitive and requires further sequencing (Fuhrman & Hagström, 2008). In a recent paper, we have investigated the interest of combining CE-SSCP fingerprinting with clone library to study the spatial distribution of phylogenetically identified bacterial populations (Rodriguez et al., in press). We underlined the fact that this possibility is restricted to some peaks, since co-migration has been observed for around half of the peaks for a given sample. Moreover, we can expect that the coupling between CE-SSCP profile to a clone library from the same sample may not be extrapolated to another CE-SSCP profile (especially if these samples have been sampled at 5 m depth and at 1000 m depth). This is particularly true in our study because of the clear changes observed in bacterial community structure with depth. However, such approach has been used by Morris et al. (2005; cited by the reviewer; assignation of T-RFLP peaks from 5 m and 200 m depth samples taken during 5 years by a sole clone library from 200 m depth at one time) or by other authors cutting bands on DGGE gels (see for example Casamayor et al., 2002), but the recent interest on co-migration phenomena in the literature may criticized this approach. Such limitation would imply to use different clone libraries from different depths, with the limitations explained above.

(ii) the use of clone libraries from different depths to unravel the physico-chemical and biological driving forces of bacterial community changes in the water column. We agree that clone libraries from different depths would have been of interest, especially in providing information about vertical distribution of ecotypes of certain phylogenetic taxa (Morris et al. 2005, Johnson et al. 2006). However, statistical analysis needs the comparison of sufficient data to be relevant. We have decided to dedicate our efforts to the comparison of bacterial community structure of numerous samples (10 depths between 5 and 1000 m at four dates) together with the corresponding data recorded at this date in the DYNAPROC-II cruise (pressure, temperature, conductivity, oxygen, salinity and photosynthetically active radiation, nitrate, nitrite, phosphate, silicate, ammonia, DOC, CDOM, twelve classes of lipids biomarkers, and six key chlorophyll and

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carotenoid pigments). There is a general agreement that genetic fingerprints are more adequate tools to study spatial and temporal dynamics of bacterial populations than the more time-consuming cloning (inventory) approaches. Because molecular inventories are limited by the number of clones analyzed, they present a large diversity with a low level of dominance and numerous singletons, whereas the images generated from a large number of 16S rDNA molecules in fingerprinting patterns present a high level of dominance (Kemp & Aller, 2004). The result given by fingerprinting techniques certainly does not represent the total species richness in the samples, but rather a standardized measure of richness (Fuhrman & Hagström, 2008). The analysis of fingerprints has been recently improved by the conclusions given by numerical simulation (Loisel et al., 2006). This study indicates that interpretation of fingerprinting based on discrete peaks (or bands) does not use the potential of fingerprinting patterns which has the potential to reveal the overall diversity of microbial ecosystems. Based on the co-migration phenomena (see description above), these authors explained that the area under a peak contains information that is relevant for microbial diversity measurement. They conclude that “contrary to molecular inventories, fingerprinting patterns do not reflect only a tiny fraction of reality but correspond to a representation of the whole consortium. […] this picture can be used to estimate microbial diversity but not by reference to the number of visible bands or peaks” [by taking into account the entire signal, including the area under the bands or peaks]. In our study, and as depicted in the material and method section (page 2140, line 28 to page 2141, line 4), we have used “the software SAFUM (Zemb et al., 2007), which renders a profile of fluorescence intensity as a function of retention time per sample taking therefore into account the presence and intensity of each individual signal”. Such treatment of fingerprint data is in accordance with the analysis of molecular fingerprinting proposed by Loisel et al. (2006).

Finally, and based on this discussion, we propose to remove the end of the sentence underlined by the reviewer (“…and presents similar or higher detection limits than DGGE or T-RFLP fingerprinting techniques (Hong et al., 2007; Smalla et al., 2007)”

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page 2135 lines 11-12), in order to avoid getting into too much of the detail that is very abundant in the literature. We also propose to add new information about CE-SSCP: Page 2135, line 14: "In a recent paper (Rodriguez et al., in press), we show that CE-SSCP do not miss any major known group detected by a clone library of the same sample and that this technique is a reasonably quantitative measure of the relative abundance of the only taxon we could independently measure (Prochloroccocus, by flow cytometry)." Page 2147, line 9: "Molecular fingerprinting techniques in general may be restricted in giving information about microbial diversity, since they result in 30 to 40 peaks (or bands) in marine waters. This is due to co-migration of PCR-products from different species within the same peak (or band), as depicted by several authors (Wintzingerode et al., 1997; Schmalenberger and Tebbe, 2003). Similarly, Schauer et al. (2000) mentioned the subjectivity in deciding whether a very weak DGGE band is a real band or a background artefact. In our study, this problem was, at least partly, overcome by taking into account the presence or absence of individual CE-SSCP peaks and the relative contribution of each peak to the total surface area of the pattern. The advantage of analysis fingerprinting data this way has been recently enlightened by numerical simulation (Loisel et al., 2006). This study underlines that fingerprinting patterns contains extractable data about diversity although not on the basis of a number of bands or peaks, as is generally assumed to be the case, and can be considered as an "image" of the whole microbial ecosystem free of inventory (cloning) limitation."

Reference to be added in the revised version of the manuscript: Loisel, P., Harmand, J., Zemb, O., Latrille, E., Lobry, C., Delgenès, J.P., and Godon, J.J. : Denaturing gradient electrophoresis (DGE) and single-strand conformation polymorphism (SSCP) molecular fingerprintings revisited by simulation and used as a tool to measure microbial diversity, Environ. Microb., 8, 720-731, 2006.

Rodríguez-Blanco, A., Ghiglione, J.F., Catala, P., Casamayor, E.O., and Lebaron, P.: Spatial comparison of total vs. active bacterial populations by coupling genetic finger-

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printing and clone library analyses in NW Mediterranean Sea, FEMS Microb. Ecol., in press.

Schauer, M., Massana, R., Pedrós-Alió, C.: Spatial differences in bacterioplankton composition along the Catalan coast (NW Mediterranean) assessed by molecular fingerprinting, FEMS Microbiol. Ecol., 33, 51-59, 2000.

Schmalenberger, A., and Tebbe, C.C.: Bacterial diversity in maize rhizospheres: conclusions on the use of genetic profiles based on PCR-amplified partial small subunit rRNA genes in ecological studies, Mol. Ecol., 12, 251-261, 2003.

Wintzingerode, F.V., Göbel, U.B., and Stackebrandt, E.: Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis, FEMS Microbiol. Rev., 21, 213-229, 1997.

Specific comment 3: We agree with the reviewer"s remark and we propose to add information about "bottom-up" and "top-down" control in the introduction and in the discussion sections: Page 2134, line 21: "More generally, theoretical models and empirical surveys indicate that microbial growth or population size are controlled by resource availability, including nutrient and energy sources ("bottom-up" control) and microbial biomass is controlled by mortality, including factors such as predation or viral lysis ("top-down" control) (Pernthaler, 2005). The relative importance of "top-down" and "bottom-up" controls on bacteria have been poorly investigated (Fuhrman and Hagström, 2008), and our knowledge on the interplay between the different factors involved within "top-down" and "bottom-up" controls is still limited in the field. Protistan predation may often be most influential in limiting the total abundance and biomass of the bacterioplankton, whereas viruses are considered to more profoundly affect prokaryotic community diversity (Pernthaler, 2005). On the other hand, clear bottom-up effects on bacterial community structure have been observed in controlled laboratory conditions (Pinassi et al., 1999; Carslon et al., 2002) but few studies demonstrated statistically robust, predictable patterns in the field (Korona et al., 1994). Such complex ecological ques-

tions may be addressed by robust statistical analysis. A recent review (Ramette, 2007) underlines that hypothesis-driven technique such as redundancy analysis, canonical correspondence analysis (CCA), or Mantel tests are rarely used by microbial ecologists, despite the fact that high-throughput molecular technologies are now available at the field. For example, this approach allows better understanding of the linkage between bacterioplankton and phytoplankton dynamics in marine coastal environment (Rooney-Varga et al., 2005)."

We propose to remove section page 2134, lines 21-27.

Page 2152, line 5: "In our conditions, resources ("bottom-up" control) as well as other factors such as pressure, temperature, light, O2 and PAR controlled simultaneously the vertical zonation of bacterial communities in the water column. Unfortunately, the parameters measured in our study do not permit to evaluate the effect of predation by viruses or protists ("top-down" control). There are indications in the literature that bacteria are tightly controlled by protistan predation in highly oligotrophic systems, whereas their growth is limited by competition for nutrients in more productive waters (Strom, 2000; Gasol et al., 2002). On the other hand, virus-induced mortality seems more substantial when bacterial productivity is enhanced or in anoxic conditions (Weinbauer et al., 2004). "Sideways controls" such as competition for resource, allelopathy or syntrophy may also influence bacterial community structure in marine environments and the new insights given by metagenomics approach should allow more thorough investigation of this subject in the near future (Fuhrman and Hagström, 2008; Strom et al., 2008). Numerous factors can regulate microorganism population dynamics, often simultaneously, and our study together with other evidences found in the literature (Fuhrman et al., 2006) underline the importance of relevant statistical analysis for further multidisciplinary investigations to investigate the relative importance of environmental factors in predicting the bacterial community dynamics in marine systems."

We propose to remove section page 2152, lines 5-8.

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References to be added in the revised version of the manuscript:

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Fuhrman, J.A., and Hagström Å.: Bacterial and Archaeal community structure and its patterns. In: Microbial Ecology of the Oceans (2nd edition), edited by Kirchman, D. L., Wiley-Liss, Inc., New York, 45-90, 2008.

Fuhrman, J.A., Hewson, I., Schwalbach, M.S., Steele, J.A., Brown, M.V., and Naeem, S.: Annually reoccurring bacterial communities are predictable from ocean conditions, Proc. Natl. Acad. Sci., USA, 103, 13104-13109, 2006.

Gasol, J. M., Pedros-Alio, C., and Vaqué, D. Regulation of bacterial assemblages in oligotrophic plankton systems: results from experimental and empirical approaches. Antonie Van Leeuwenhoek, 81, 435-452, 2002.

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Pernthaler, J.: Predation on prokaryotes in the water column and its ecological implications, Nature, 3, 537-546, 2005.

Pinhassi, J., Azam, F., Hemphälä, J., Long, R.A., Martinez, J., Zweifel, U.L., and Hagström, Å.: Coupling between bacterioplankton species composition, population dynamics, and organic matter degradation. Aquat. Microb. Ecol., 17, 13-26, 1999.

Rooney-Varga, J.N., Giewat, M.W., Savin, M.C., LeGresley, M., and Martin, J.L. Links between phytoplankton and bacterial community dynamics in a coastal marine environment. Microb. Ecol., 49, 163-175, 2005.

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Weinbauer, M.G., and Rassoulzadegan, F.: Are viruses driving microbial diversification and diversity? Environ. Microbiol. 6, 1-11, 2004.

The vertical distribution of bacterial communities has been discussed in more details with regards to the reviewer comments. Page 2148, line 3: This is also consistent with genotypic and phenotypic properties of stratified Prochlorococcus "ecotypes" for example, that are suggestive of depth-variable adaptation to light intensity and nutrient availability (Rocap et al., 2003). However, none of these studies tested for the statistical significance of vertical trends (autocorrelation), nor did they test for the predictability of vertical pattern from environmental parameters, so we do not know whether these studies show spatial patterning or predictability comparable to what we observed. Recent metagenomic approaches showed that vertical zonation of taxonomic groups was concomitant with functional gene repertories and metabolic potential (DeLong et al., 2006) and future efforts are needed to interpret the observed genomic pattern as a function of contextual environmental parameters (Ramette, 2007).

References to be added in the revised version of the manuscript: DeLong, E.F., Preston, C.M., Mincer, T., Rich, V., Hallam, S.J., Frigaard, N.U., Martinez, A., Sullivan, M.B., Edwards, R., Brito, B.R., Chisholm, S.W., and Karl, D.M.: Community genomics among stratified microbial assemblages in the ocean's interior. Science, 311, 496-503, 2006.

Rocap, G., Larimer, F.W., Lamerdin, J., Malfatti, S., Chain, P., Ahlgren, N.A., Arellano, A., Coleman, M., Hauser, L., Hess, W.R., Johnson, Z.I., Land, M., Lindell, D., Post, A.F., Regala, W., Shah, M., Shaw, S.L., Steglich, C., Sullivan, M.B., Ting, C.S., Tolonen, A., Webb, E.A., Zinser, E.R., Chisholm, S.W.: Genome divergence in two Prochlorococcus

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ecotypes reflects oceanic niche differentiation. Nature, 424, 1042-1047, 2003.

Specific comment 4: The primers used in this study amplify the variable V3 region of the 16S rDNA (Escherichia coli positions 329-533) and they were designed to amplify Eubacteria (voir Delbès et al., 2000 and et Lee et al., 1996) but not Archaea. References of each primer were provided in the original manuscript (Delbès et al., 2000 and Lee et al., 1996).

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Horz, H.P., M. Tchawa Yimga and W. Liesack, Detection of methanotroph diversity on roots of submerged rice plants by molecular retrieval of pmoA, mmoX, mxaF, and 16S rRNA and ribosomal DNA, including pmoA-based terminal restriction fragment length polymorphism profiling, Appl. Environ. Microbiol. 67 (2001), pp. 4177-4185.

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Hong, H., Pruden, A., and K.F. Reardon. (2007) Comparison of CE-SSCP and DGGE for monitoring a complex microbial community remediating mine drainage. Journal of Microbiological Methods, 69 (1): 52-64.

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