Letters

Bulk Extraction-Based Microbial Ecology: Three Critical Questions

The use of bulk extraction to recover nucleic acids from soils, waters, and other environments for studies of 16s rRNA/DNA and metagenomics-based molecular biology continues to gain in prominence. In addition, bulk extraction is being used to recover nucleic acids for studies of plant and animal "microbiomes." At the most recent ASM meeting, held in New Orleans in May 2011, based on my review of the abstract titles, there were a total of at least 19 sessions where microbial or bacterial "communities" were studied, based primarily on the use of bulk-extracted nucleic acids. This approach is used widely in the current literature, where microbial diversity and microbial communities are claimed to be studied (as examples, Web of Science[SM] shows 1516/3979 citations linking 16s rDNA/RNA and community, 2,326/4,980 citations linking 16s rDNA/RNA and microbial diversity, 400 citations where metagenomics and community are linked, 323 citations where metagenomics and microbial community are linked, and 253 citations where metagenomics is linked with microbial diversity). In addition, sophisticated kits now are available (check advertisements in recent issues of *Microbe*) that allow one to recover nucleic acids from soils and other environments by use of bulk extraction and which contain necessary reagents and procedural information to generate molecular sequences that are ready for computer-based analysis and uploading into publications. The most critical part of this process, the bulk extraction-based recovery of nucleic acids from the experimental matrix, usually is simply noted as "DNA is/was extracted," with no further information provided.

In my view, there are at least three critical questions that need to be considered when bulk-extracted nucleic acids are used in these studies. (i) Using a bulk extraction-based approach for the recovery of nucleic acids from natural microbial assemblages, does one know the source(s) of the nucleic acids that have been recovered? (ii) Have any of the recovered nucleic acids been derived from extracellular sources? (iii) If it is claimed that microbial cells , or more specifically in situ active cells, the microbial community, are being studied, has it been proven that microbial cells, whether inactive or active, respectively, are the sole sources of the recovered nucleic acids?

When this research approach was first developed in 1985, based on my reading of this literature (N. R. Pace, D. A. Stahl, et al., *ASM News* **51:**4–12, l985), these questions had not even been considered. Since that time, it has become evident that natural microbial assemblages contain nucleic acids that are derived from a variety of sources, including nucleic acids that are not associated with cells, or more specifically not associated with in situ active cells (D. A. Klein, *Microbe*, December 2007, p. 591–595). As a particularly important point, significant quantities of extracellular nucleic acids occur in natural environments, whether soils (P. Cai, Q. Y. Huang, et al., Pedosphere **15:**16–23, 2005; G. Pietramellara, J. Ascher, et al., Biol. Fert. Soils **45:**219–235, 2009), freshwater (J. H. Paul, W. H. Jeffrey, et al., Appl. Environ. Microbiol. **56:**2957–2962, 1990), marine environments (A. Dell'Anno and R. Danovaro, Science **309:**2179, 2005), and particularly biofilms (R. E. Steinberger and P. A. Holden, Appl. Environ. Microbiol. **71:**5404–5410, 2005). This extracellular/environmental DNA can be PCR amplified (A. J. Alvarez, M. Khanna, et al., Mol. Ecol. **7:**775–778, 1998; U. Böckelmann, A. Janke, et al., FEMS Microbiol. Lett. **262:**31–38, 2006) and can be included in metagenomic analyses (J. F. Petrosino, S. Highlander et al., Clin. Chem. **55:**856–866, 2009). In light of the widespread occurrence of such extracellular nucleic acids, it does not appear to be defensible to note that nucleic acids are being recovered solely from "microbial cells" using this approach, as has been assumed in a recent publication (V. A. Kunin, A. Copeland, et al., Microbiol. Mol. Biol. Rev. **72:**557–578, 2008), where no proof is provided that microbial cells would be the sole source of the recovered nucleic acids. The study of "microbial communities" using bulk-extracted nucleic acids raises similar questions; in most papers where microbial communities purportedly have been studied, no proof has been provided that the recovered nucleic acids, derived by the use of bulk extraction-based approaches, actually were derived specifically from in situ active microbes. This experimental point was discussed recently (D. A. Klein, *Microbe*, May 2010, p. 189).

These questions have been discussed in recent blogs (http://schaechter.asmblog.org/schaechter/page/26/; http://schaechter.asmblog.org/schaechter/2010/01/an-open-invitation-to-argue-with-me.html#comments). As discussed in these blogs, in using bulk extraction-based approaches to recover nucleic acids from natural microbial assemblages, conclusions too often are drawn concerning the source(s) of these nucleic acids without providing rigorous proof concerning their actual source(s). In my opinion, the time is long overdue to confront these questions, concerning the source(s) of nucleic acids recovered from natural microbial assemblages by bulk extraction-based procedures.

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