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# Is There Anything Else You Need To Understand About the Microbiota That Cannot Be Derived from Analysis of Nucleic Acids?

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Larry Pomeroy's insight into the importance of the pelagic marine microbial community has been more than validated by recent work that goes far beyond the classical techniques of culture and microscopic analysis by epifluorescence microscopy. Two manuscripts in this volume summarize the utility of nucleic acid analysis for determining microbial community structure [2] and measuring growth and cell division [7].

Will nucleic acid analysis provide all you need to know about the pelagic microbial community? First, in the matter of determining microbial community structure, nucleic acid analysis of the pelagic marine community has put to rest one problem that plagues microbiologists: the idea that if an organism cannot be cultured, it is not present. The detection of a taxonomically diverse nanoplankton community in the sea by rRNA analysis that eluded cultural techniques demonstrated the power of this technology. The ability to amplify nucleic acids with the polymerase chain reaction (PCR) and the relative ease of extracting nucleic acids from seawater (without tannins or other PCR inhibitors) makes community analysis by rRNA even more valuable.

But is nucleic acid analysis enough? My brain cells and kidney cells have exactly the same DNA and ribosomal rRNA, yet they perform completely different functions. Measurement of mRNA would be helpful but still would not completely answer the question of gene expression, as sometimes mRNA is not translated or the enzymes formed are not functional when subjected to posttranslational control.

Can measurement of DNA synthesis by thymidine incorporation (or possibly more inclusively by $H_3{}^{32}PO_4$ incorporation) indicate metabolic activity? My neurons are not dividing and again I hope they are metabolically active! In bacteria, Poly β-hydroxyalkanoate (PHA) synthesis goes on rapidly and can represent significant metabolic activity and biomass increase under specific conditions when there is no DNA synthesis.

So, the problem of what is going on at the moment with the microbes is much more complex than nucleic acid analysis is capable of answering, and requires unequivocal phenotypic information. A complementary technique that provides this phenotypic information involves signature lipid biomarker (SLB) analysis.

The SLB analysis involves a one-phase solvent extraction, fractionation of the lipids on silicic acid, derivatization, and analysis by gas chromatography/mass spectrometry [3, 13]. Samples of microbial slimes, sediments, soils, bioreactors,

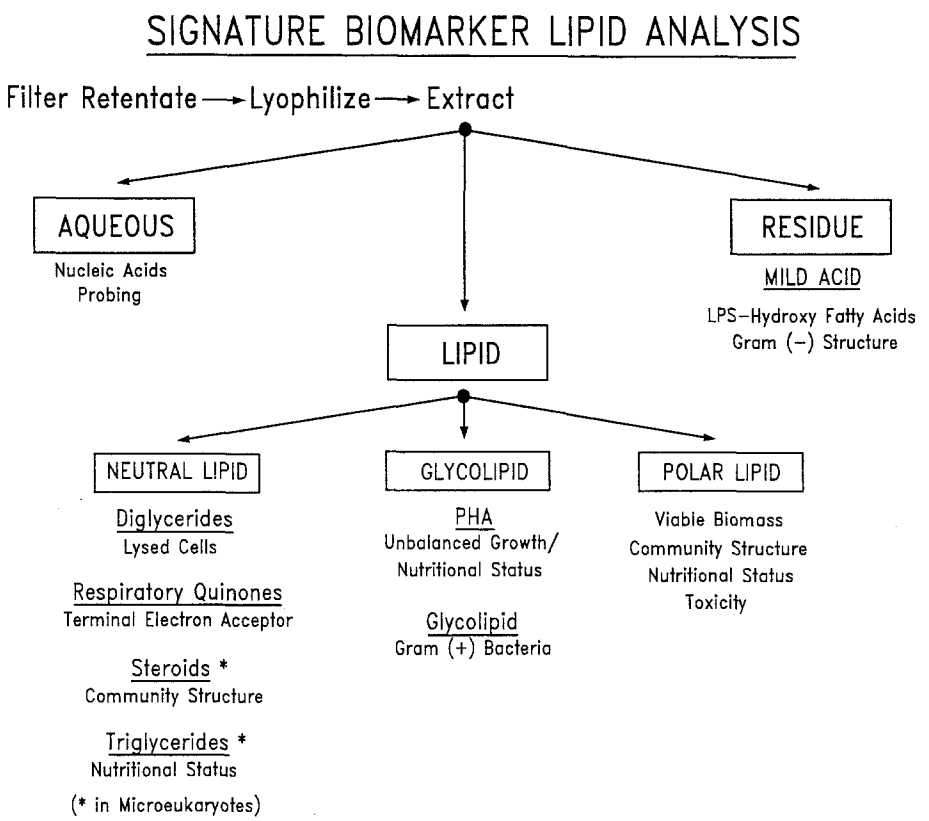


**Fig. 1.** Scheme for the signature lipid biomarker (SLB) technique in which membrane filter retentates are extracted, the nucleic acids recovered and probed, the lipids fractionated, and the fractions analyzed by gas chromatography/mass spectrometry after derivatization.

the rhizosphere, sea ice, indoor air, and membrane filter retentates have been analyzed [11, 12], providing insight into the viable biomass, the total biomass, the community structure, and the nutritional/physiological status of the communities (Fig. 1). Validation of the SLB analysis has been reviewed [12].

Viable microbes have an intact membrane that contains phospholipids and phospholipid ester-linked fatty acids (PLFA). With cell death, enzymes hydrolyze the phosphate group within minutes to hours [13]. The lipid core remains for some time as diglyceride (DG). The resulting DG has the same signature fatty acids as the phospholipids (until it degrades), so a comparison of the ratio of PLFA to DG provides insight into the proportions of viable and lysed microbes. Gassing subsurface sediments with methane or propane induces growth of type II methane-oxidizers and/or actinomycetes [8]. Rates of formation of specific metabolic products in estuarine sediments correlate with specific types of sulfate-reducing bacteria as determined by SLB analysis [10], as specific groups of microbes contain characteristic PLFA patterns. Conditions favoring fungal colonization of detritus correlate with increases in specific steroids [11]; specific anaerobic aryl dehalogenating bacteria can be detected in situ in sediments by their signature lipopolysaccharide

hydroxy fatty acids [9]. Not only can the SLB analysis provide quantitative definition of community structure but the community nutritional status can be determined. Bacteria undergo unbalanced growth and form PHA if some essential component is missing and adequate carbon and terminal electron acceptors are available [11, 12]. Specific patterns of PLFA can indicate physiological stress [4]. Exposure to toxic environments can lead to minicell formation and shifts in PLFA. Formation of increasing proportions of *trans* fatty acids with exposure to increasing concentrations of phenol toxicants has been shown for *Pseudomonas* [6]. Respiratory quinone structure indicates the proportions of aerobic, anaerobic respiratory, or anaerobic fermentative growth in the community. It was possible to show that anaerobic respiratory metabolism was responsible for siderite formation in estuarine concretions utilizing SLB analysis [1].

The major limitations of SLB analysis are that at least $10^7$ cells are required and that analysis by solvent extraction is time consuming (25 analyses per week per person). The sensitivity is greatly increased with electron withdrawing derivatization and negative ion detection mass spectrometry [14]; replacement of the solvent extraction with a semi-automated supercritical fluid extraction greatly speeds up the analysis [5]. The SLB analysis and nucleic acid probing are complementary in that the extraction process can be modified to also yield nucleic acids for molecular analyses from the same sample (Fig. 1).

Signature lipid biomarker (SLB) analysis is an excellent complement to nucleic acid analysis (for specific gene frequencies) in that it readily provides a comprehensive phenotypic description of the in situ community in environmental samples.

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