OIKOS 74: 177–184. Copenhagen 1995

Chemical ecology: possible linkage between macro- and microbial ecology

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White, D. C. 1995. Chemical ecology: possible linkage between macro- and microbial ecology. – Oikos 74: 177–184.

Microbes are often the largest biomass in an ecosystem, yet are not often considered in classical ecology. Problems with the study of the ecology of microbes come from the difficulties in applying the isolation and culture techniques that were so successful in medical studies but are not effective in the environment. Most environmental microbes simply will not grow in culture. The problem of assessing the impact of microbes on ecosystems is further complicated as the most significant impact of a microbial community on an ecosystem is the result of metabolic activities. Inactive microbes can be a food source, but the biogeochemical metabolism of the active microbes have the greatest impact on the environment. In stable soils and sediments, only a tiny proportion of the potential metabolic capacity and versatility of that community is being actively expressed at a given time. Microbes in these environments are poised to rapidly take advantage of nutrients that become available following a disturbance. This dramatic reaction to disturbance makes measuring the metabolic activity of microbes in undisturbed soil a significant challenge. Heterogeneity on the scale of the microbes in the distribution of nutrients, carbon sources, water, ions, or terminal electron acceptors in soils is a significant complication. The assumptions of colligative properties of solutions for soil environments in the estimations of kinetics is one of the reasons that engineers have so much trouble making successful models for in situ bioremediation in these heterogeneous soils and sediments. How then, can soil microbial community ecology be approached? The hypothesis that many of the responses of monocultures of microbes to specific conditions will predict the responses of these organisms to specific conditions in the soil community seems to hold true. This theory is being tested using genetically engineered microbes with "reporters" in biofilms and can be examined in soils, if the analysis does not apply selective pressures that can distort the in situ conditions. The knowledge of physiological responses of monocultures, and the structural modifications they produce, provides a mechanism to predict the responses of a community to specific change. In characterizing the microbial community in situ, there are advantages to analyzing lipid membranes, because all viable cells are enclosed in lipid membranes containing polar lipids. If specific organisms have sufficiently unusual lipids in their membranes then these lipids can serve as "signatures" to define the community structure. If there are specific conditions that result in structural modifications of the lipids, then the detection of these modifications can be utilized to define the ecology of the microniche of that organism and thus of at least a portion of the microbial community. This gives insight into the microbes' nutritional/physiological status and the suitability of the local environment. The presence of polar lipids can define the viable biomass, as no cell can function without an intact membrane which contains polar lipids. The activation of endogenous phospholipase activity with cell death can leave traces of the recent lysis in the diglyceride structure. The detection of specific genes can define the limits of adaptation but not always reflect current metabolic activity. That most complex ecosystem with the most diverse biota and versatile biochemistry, soil, is finally approachable on both a holistic and utterly reductionest scale, thanks in large measure to the prescient insight of Per Brinck who introduced Chemical Ecology to Lund University and the rest of the world.

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Accepted 16 March 1995. This is a revised version of David White's 1994 Per Brinck lecture

ISSN 0030-1299

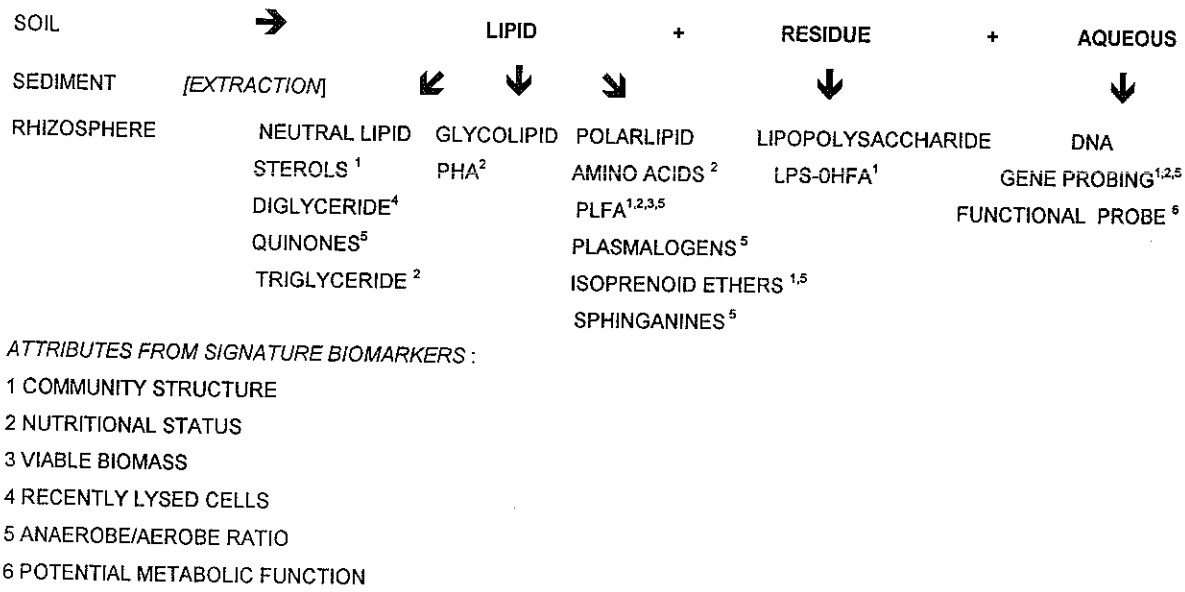

Fig. 1. Attributes of microbial communities based on Signature Biomarker Analysis (Lipids and DNA).

The problem

Many ecologists know in their hearts that the unseen microbes in their ecosystem must be very important. After all they comprised the biosphere for almost all but the last 6.0×10^8 of 3.8×10^9 yr that life existed on the earth and are responsible for the geochemical cycles that allow life to continue. Microbes tend to be ignored because they are difficult to study. The classical methods of isolation and culture of microbes that are taught in most microbiology courses do not work when applied to the environment as less than 1% of what can be detected in stained microscopic preparations can be cultured. Staining microbes in soils can be difficult as many are attached to soil granules and can be hidden. Agents that release attached microbes are often selective and do not release quantitatively. The morphology of the microbes does not often reflect the function or activity so very little insight into the community structure or nutritional status is possible. Measurements of metabolic processes are complicated by the facts that most microbes in the soil are inactive, but poised for activity when nutrients appear. Adding labeled substrates to determine rates of metabolic activity induce major disturbance artifacts giving much higher rates than actually exist in the environment. This is possibly best exemplified in studies of the deep subsurface microbiota where oxygen and inorganic carbon are found in groundwater with a ground water age of greater than 1.1×10^5 yr. Measurements of metabolic activity based on isotope incorporation experiments by the microbes in subsurface sediments were 10^3 to 10^6 times greater than the geochemical evidence would predict. The metabolic activities by the subsurface microbiota indicate growth rates of centuries (Phelps et al. 1994).

The challenge of including the microbiota in ecosystems has captured some of the most perceptive ecologists. The great "synthesizer" E. O. Wilson of Harvard University in his wonderful autobiography "Naturalist" stated "If I could do it all over again, and relive my vision in the twenty-first century, I would be a microbial ecologist. ...Into that world I would go with the aid of modern microscopy and molecular analysis. I would cut my way through clonal forests sprawled across grains of sand, travel in an imagined submarine through drops of water proportionately the size of lakes, and track predators and prey in order to discover new life ways and alien food webs".

Has the "methods limited" nature of microbial ecology been solved with the molecular revolution? DNA can be recovered from soils and genes probed for specific functional activities and organisms can be identified at the kingdom, family, genus, species levels based on sequence homology with various portions of the DNA coding for the ribosomal RNA's (Ward et al. 1992). However, the presence of a gene for the rDNA indicates an organism is there only if the gene is demonstrated to be in the specific organisms and not extracellular. For an enzyme activity the presence of a gene indicates that the potential exists in that ecosystem for that activity. As has been pointed out "My brain and my kidney have exactly the same sequence in the 3×10^9 bases in their DNA and in the 1.8×10^4 bases in the mitochondrial DNA I got from my mother, but I certainly hope they are not doing the same thing" (White 1994). The molecular revolution provided microbial ecology with a new an powerful technology for specific insights into the metabolic potentialities of a community. The concomitant application of Chemical Ecology can provide insights into the "phenotypes" to

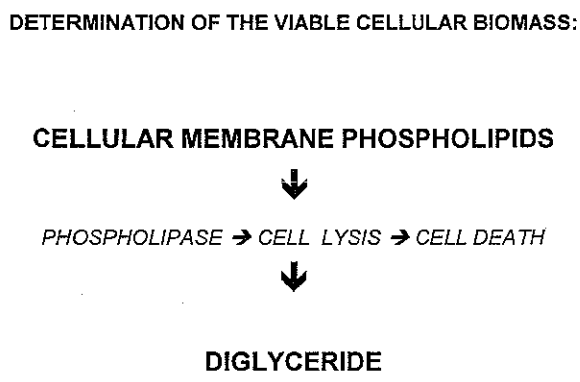

Fig. 2. Determination of viable biomass based on the analysis of the phospholipid fatty acid content. With cell lysis and death, endogenous phospholipases hydrolyze the phosphate-containing portion of the lipids forming diglyceride from the phospholipid. The resulting diglycerides retain the same fatty acid patterns of the parental phospholipids for some time.

complement the "genotypes" within the microbial community.

A possible solution

Analysis of the cellular lipids provides a satisfactory way to gain insight into critical attributes of microbial communities. Lipids are cellular components recoverable by extraction in organic solvents, and the extraction provides both a purification and concentration. Lipids are an essential component of the membrane of all cells and play a role as storage materials. The signature lipid biomarker (SLB) analysis provides quantitative insight into three important attributes of microbial communities and allows recovery of the DNA (Fig. 1).

A) Viable biomass

The determination of the total phospholipid ester-linked fatty acids (PLFA) provides a quantitative measure of the viable or potentially viable biomass. Viable microbes have an intact membrane which contains phospholipids (and PLFA). The cellular enzymes hydrolyze (release) the phosphate group within minutes to hours of cell death (White et al. 1979). The lipid remaining is diglyceride (DG) (Fig. 2). The resulting DG contains the same signature fatty acids as the phospholipids, allowing a comparison of phospholipid fatty acids to diglyceride fatty acids (viable to non-viable microbes) to be made. Since the same fatty acids present in the phospholipids are found in the diglycerides (until they are hydrolyzed), the patterns of the DG fatty acids can indicate the recently lysed components of the microbial community. A careful study of subsurface sediment showed the viable biomass determined by PLFA was equivalent (but with a much smaller standard deviation) to that estimated by intercellular ATP, cell wall muramic acid, and very carefully done acridine orange direct counts (AODC) (Balkwill et al. 1988).

B) Community structure

The analysis with SLB provides a quantitative definition of the microbial community structure. Specific groups of microbes often contain unusual lipids (White et al. 1978, White 1988, Tunlid and White 1991). For example, specific PLFA are prominent in the hydrogenase-containing *Desulfovibrio* sulfate-reducing bacteria, whereas the *Desulfobacter* type of sulfate-reducing bacteria contain distinctly different PLFA (Edlund et al. 1985, Dowling et al. 1986). Patterns of the prominent PLFA from isolated microbes after growth on standardized media are used to differentiate over 8000 species of organisms with the Microbial Identification System (MIDI, Newark DE) (Welch 1991). Hierarchical cluster analysis of the patterns of phospholipid fatty acids shows similarities between species of isolated methane-oxidizing and sulfate-reducing bacteria that almost exactly parallel the phylogenetic relationships based on the sequence similarities of the 16S rRNA (Guckert et al. 1991a, Kohring et al. 1994). Hierarchical cluster analyses of PLFA patterns of the total microbial communities quantitatively define relatedness of different microbial communities. This has been done with deep subsurface sediments in which the microbial communities of permeable strata are different from surface soils, the clay aqualudes, and the drilling fluids used to recover the samples (White et al. 1991).

The analysis of other lipids such as the sterols (for the microeukaryotes – algae, protozoa) (Nichols et al. 1990), glycolipids (phototrophs, gram-positive bacteria), or the hydroxy fatty acids in the lipopolysaccharide (LPS) Lipid A (gram-negative bacteria) (Parker et al. 1982) can provide more detailed community structure analysis (Fig. 1).

C) Nutritional status

The formation of poly β-hydroxyalkanoic acid (PHA in bacteria) (Nickels et al. 1979, Findlay and White 1983), or triglyceride (in microeukaryotes) (Gehron and White 1982) relative to the PLFA provides a measure of the nutritional status. Bacteria grown with adequate carbon and terminal electron acceptors form PHA when they cannot divide because some essential component is missing (phosphate, nitrate, trace metal, etc.). Furthermore, specific patterns of PLFA can indicate physiological stress (Guckert et al. 1986). Exposure to toxic environments can lead to minicell formation and a relative increase in specific *trans* monoenoic PLFA compared to the *cis* isomers. It has been shown that for increasing concentrations of phenol toxicants, the bacteria *Pseudomonas pudita* forms increasing proportions of *trans* PLFA (Heipieper et al. 1992).

D) Concomitant DNA extraction

Extraction of DNA from soils can be difficult. Polyphenols, tannins, and clays all make recovery amplification, and detection by DNA:DNA hybridization difficult. Where enzyme action is required in the analysis as in the amplification by Taq polymerase in the PCR or restriction digestion the concurrent extraction of enzyme inhibitors

is a serious problem. Despite the major efforts of several laboratories the most quantitative recoveries have been restricted to environments without tannins, clays, or enzyme inhibitors such as thermal spring microbial mats or pelagic seawater communities (Ward et al. 1992). Recent evidence indicates that the lipid extraction used for SLB analysis also liberates the cellular DNA (Kehrmeyer et al. unpubl.). Over 99% of ^{32}P-labeled DNA added to soil was recovered in the aqueous phase of the lipid extraction. Over 50% of the gene *nahH-lux* added in intact *Pseudomonas fluorescens* to the soil recovered by the standard techniques (Ogram et al. 1987) was recovered in the aqueous phase of the lipid extraction (Kehrmeyer et al. unpubl.). The DNA recovered from the lipid extraction was amplified successfully in the polymerase chain reaction (PCR).

A powerful quantitative assessment method developed over the past 20 yr by this laboratory to define the viable biomass, community structure and nutritional/physiological status of environmental microbial communities based on signature lipid analysis can be expanded to include defining the proportions of specific genes or microbial species based on the extracted DNA. The DNA probe analysis offers powerful insights because of the exquisite specificity in the detection of genes for enzyme processes, 16S rRNA for organism identification at the kingdom, family, genus or species levels. One problem with genetic analysis is that it is difficult to achieve quantitative results. The concomitant DNA/lipid analysis readily provides quantitative recoveries independent of the ability to isolate or culture the microbes, and the presence of intact cellular membranes containing polar lipids provides an accurate measure of the microbial biomass as when compared to intercellular ATP, muramic acid, endotoxin, and direct microscopic count in a subsurface sediments (Balkwill et al. 1988). With gene probes it is relatively easy to compare the relative proportions of specific potential functional genes or specific organisms to "all bacterial" sequences in the 16S rRNA and calculate the biomass from the lipid analysis. The second advantage of the combined SLB/DNA analysis comes from the fact that having detected the gene gives only the potential for its activity. The lipid analysis gives indications of the physiological status that can indicate activity for the enzymes detected as present by the gene probes. Most enzymes are not active at any given time (White 1994). The SLB analysis provides in situ indications of starvation, growth rate, exposure to toxicity, unbalanced growth, phosphate availability, microniche pH, moisture, the aerobic/anaerobic metabolic balance. The components and proportions of the recently lysed (dead) microorganisms provide significant insight into the phenotypic activity of the communities. These provides the basis for better predicting the metabolic activity of the genes detected with the probes. Recently DNA recovered from the aqueous phase of the lipid extraction (Kehrmeyer et al. unpubl.). Since SLB has been applied successfully to the rhizosphere and to soils (Tunlid et al. 1985, Tunlid and White 1991), concomitant SLB and DNA gene probe analysis of soils and rhizospheres should provide a powerful new analysis system for determining ecology and community dynamics of the microbiota.

Soil and rhizosphere microbial diversity is still largely undefined (Aldhous 1994). The combined SLB/DNA assay system provides a quantitative baseline and a possible holistic analysis scheme.

We are still left with the problem of determining metabolic activity of a community poised for rapid acquisition of any resources presented by disturbance. Chemical ecology and the SLB analysis provides a quantitative means to measure the effects of disturbance in sediments where an aerobic surface overlies an anaerobic sediment rich in reduced substrates. Disturbance introduces aerated water to the anaerobic sediment and a burst of activity ensues with rapid growth until a critical nutrient again limits cell division. However carbon accumulation continues without cell division and new cell membrane synthesis. This is readily detectable as an initial decrease in the ratio between the rates of synthesis of PHA and PLFA followed by a rebound with PHA accumulation without PLFA synthesis. The measurement of the PHA/PLFA ratio has been shown to be an exquisitely sensitive measure of the unbalanced growth engendered by disturbance in sediments (Findlay et al. 1990a, b) or levels of impaction by pollutants on stream periphyton (Guckert et al. 1991b, Napolitano et al. 1994).

Insight without disturbance artifacts

If insight into the microniche ecology could be derived without the disturbance artifacts, a better estimate of the microbial community metabolic activity could be gained. The hypothesis that responses of specific monocultures to specific conditions will predict these responses of these organisms in communities to the same specific conditions. This hypothesis can be tested in a system utilizing non-destructive, in-line monitoring of microbial biofilm communities in a device where the biofilm is subjected to controlled laminar flow sufficient to remove pelagic microbes and provide nutrients from a dilute bulk phase. This requires a biofilm be generated on a substratum in a flow-through system that models the slimes of periphyton on rocks in a stream. If the system is outfitted with quartz windows that can be cleaned, it is possible to utilize tryptophane fluorescence of the bacterial proteins to monitor formation and biomass of the biofilm (Angell et al. 1993). The metabolic status of naturally bioluminescent *Vibrio harveyi* can be followed by comparing the fluorescence yield/cell of monocultures to various sublethal impacts (Arrage et al. unpubl.). A genetically engineered *Pseudomonas fluorescens*, containing the *lux* gene cassette for bioluminescence with a promoter that is activated by salicylate provides a reporter for bioavail-

Table 1. Community structure based on the proportions of terminal branched PLFA (found largely in gram-positive and sulfate-reducing gram-negative bacteria), polyenoic PLFA (found primarily in the photosynthetic and heterotrophic bacteria), the monoenoic PLFA (found in gram-negative heterotrophic bacteria), and the saturated PLFA (found in all organisms).

Sites[1]	Viable biomass nmol PLFA/g dry wt X, σ, number samples	% Terminal saturated PLFA	% Polyenoic PLFA	% Monoun-saturated PLFA	% Saturate d PLFA
Antarctic East	44.3, 18, n=3	5	23	55	16
Antarctic West	6.0, 2.3, n=4	6	20	49	22
Deep Sea Surface	7.9, 2.8, n=48	14	6	56	24
Deep Sea 9–10 cm	2.1, 1.0, n=20	15	1.5	39	46
Neotropical Australia	30, 12, n=20	12	8	26	52
Neotropical USA	36.3, 16.2, n=23	5	8	45	43

[1]Cape Evans East McMurdo Sound, Antarctica, top 1 cm sediment, 35 m depth + 1.5 m ice; Arthur Harbor West McMurdo Sound Antarctica top 1 cm, 26 m depth + 2.3 m ice; Benthic sea bottom top 1 cm, Scotian Rise, North Atlantic 40°27'N, 62°20'W at 4820 m depth; Same samples but 9–10 cm below sediment surface; Sand seagrass bed Moreton Bay, Queensland, Australia, top 2 cm sediment 1 m depth; Tidal sea grass estuary in the Gulf of Mexico off Florida, top 1 cm depth, 1 m depth. (Smith et al. 1986, 1989, Moriarty et al. 1985, and Baird et al. 1985).

ability of the metabolites in the salicylate pathway and a bioluminescence intensity that reflects other conditions such as oxygen availability, iron, trace metals, etc. (Heitzer et al. 1994). A similar system with a *lux* reporter with the promoter for alginate exopolymer also shows induction that parallels the detection of the exopolymer in biofilms (Rice et al. unpubl.). These "reporter" organisms have been shown to behave in mixed biofilms as they do in monocultures. Specific organisms can be detected in mixed culture biofilms if they have distinctive lipids such as *Legionella pneumophila* (Walker et al. 1993) or are tagged with the green fluorescent protein reporter (Chalfie et al. 1994) and then localized within the biofilm.

With the presumptive evidence that bacteria showing specific responses in monoculture biofilms will show many of those same responses whenever they sense the same microniche environmental conditions, it becomes possible to utilize the SLB analysis to define the nutritional/physiological status of those organisms and presumably all those in the same microniche. Some of the useful insights come from analysis of organisms like *Pseudomonas* species which form acyl-ornithine lipids when growing with limited bioavailable phosphate (Minnikin and Abdolrahimzadeh 1974). Some gram-positive bacteria form increased levels of acylamino acid phosphatidylglycerols when grown at sub-optimal acid pH levels (Lennarz 1970). Many bacteria form PHA under conditions of unbalanced growth when a carbon source and terminal electron acceptors are present but cell division is limited by lack of some essential nutrient. Aerobic growth conditions with high-potential electron donors induce facultative gram-negative heterotrophic bacteria to form respiratory benzoquinones whereas anaerobic growth conditions can induce high ratios of iso branched/antiiso branched PLFA typical of *Desulfovibrio* type sulfate-reducing bacteria or plasmalogen phospholipids typical of the *Clostridia* (White et al. 1978, 1980, White 1988, Tunlid and White 1991). Prolonged starvation or exposure to toxics such as phenols or organic solvents, induce some gram-negative bacteria to form *trans*-PLFA. Prolonged exposure to conditions inducing stationary growth phase induces the formation of cyclopropane PLFA. Cell death, with the release of endogenous phospholipases, results in the conversion of phospholipids into the diglycerides with preservation of the PLFA patterns (Fig. 2). This gives an indication of the portion of the microbial community that most recently lysed.

The insight into the environment of the microbial community is thus based on these biomarkers. This insight together with the quantitative estimation of the viable and recently lysed biomass, and functional group community structure supplemented by the gene probes for potential functional activities and specific microbes at the levels of kingdoms, genus, species based on the genes for rRNA, provides a much deeper understanding of the microbial communities.

The opportunities to correlate microbial and macroecology

Application of the SLB/DNA assays to microbial communities make it possible to pose some questions about including the microbial world in ecology.

Plant rhizosphere-microbe interactions

There are some special problems that now can be examined. The first question could possibly be solved with currently available technology. Is the present day terrestrial soil microbial ecosystem primarily a function of the rhizosphere and litter microbiota? How uniform are the plant-microbe associations? Do the same species of plants in the same field have the same rhizospheres? How widespread are the associations to different regions? Preliminary investigations show that white oaks (*Quercus alba* L.) transplanted into the same forest enclosures and grown together for three seasons maintained different

mycohorrhiza (Ringelberg et al. unpubl.). The mycorrhiza apparently reflected the initial growth conditions to which the acorns were exposed. The plant-microbe interactions represent a very fruitful area of research.

Global microbial biodiversity gradient

The SLB/DNA assays can also be utilized to ask some further questions. Is there a global biodiversity gradient in the microbial world? It is certainly clear that the microbial viable biomass based on the PLFA (including all microorganisms with an intact cellular membrane) in marine sediments are remarkably constant (Table 1).

A simplistic interpretation of major fatty acid biosynthetic pathways for short terminally branched saturated PLFA characteristic of gram-positive and anaerobic gram-negative sulfate-reducing bacteria, polyenoic PLFA characteristic of microeukaryotes, and monoenoic PLFA with unsaturation in the anaerobic desaturase pathway characteristic of gram-negative heterotrophic bacteria utilized in Table 1, gives indications of community structure. The Antarctic shows higher proportions of polyenoic PLFA and the deep sea sediments the least (Table 1). Detailed analysis of the PLFA patterns in the original publications suggests specific differences in community structure but does not indicate a clear gradient in biodiversity. Metabolic activities however, measured with injected substrates in situ, with as small a disturbance artifact as possible, showed the neo-tropical sediments to be at least 300-fold more active in terms of DNA synthesis from ^{3}H-thymidine incorporation rates than those in the Antarctic. The major global gradient appears to be in metabolic activity but not viable biomass or community structure at the grossest levels. Clearly a quantitative method for estimating microbial biodiversity is an essential component in determining if there is a global diversity gradient that matches the macroecosystem biodiversity. We are currently attempting to use a series of simple activity measures of community metabolic capability which in the more diverse communities would be expected to have much wider pH, temperature, salinity, etc., range of significant activity than the communities in the purportedly less diverse communities.

Soil microbial biodiversity

Torsvik et al. (1994) showed there must be thousands of species of microbes in the soil microbial community by determining the heterogeneity of DNA. Why is there such a species richness in the soil microbial community? In the open ocean the wide diversity of bacterial species has been attributed to an equally wide diversity of recently discovered bacteriophage in the sea (Bergh et al. 1989). This might suggest the wide diversity of soil microbiota reflects a response to predation. Could the great diversity of soil microbiota reflect the same basic response as found in the plant seed bank? In testing the plant seed bank plots of native soil set out in frames and allowed to grow produce a diverse community that is essentially unpredictable and irreproducible when soil from the same central source is utilized repeatedly. Soil microbes could possibly be analogues of plant seeds and thus governed by the same rules of evolution. Most of the time they are inactive awaiting rain or some disturbance for a burst of growth followed by a long period of inactivity. Perhaps species can accumulate because they have so little impact on the soil until the time for their activity is achieved. Determining the global gradient in microbial biodiversity reflects a true global macroecological diversity which is not the simple counting of extant plants and animals but requires evaluating the potential in the spores, seeds, and cysts as well.

Questions in evolution of life

The new technology of including the microbial world into ecology leads to other questions. Why are there multicellular eukaryotes? Multicellular eukaryotes have been a part of the biosphere for less than 17% of the 3.4×10^9 yr life has had a detectable imprint on this planet. Macro-species diversity seems to have had 4 major phases of expansion. First was the two-dimensional world of the Ediacaran which came to an end with the Cambrian expansion signaled by the appearance of predation and the development of three-dimensional benthic marine communities. Was this an accident, or the inevitable response to oxygenation of the atmosphere which allowed efficient aerobic metabolism and multicellular organisms to function? Extention to the land and increasing encephalization of predators resulted in the Paleozoic expansion of diversity which apparently built on a few of the basic body plans that appeared in the Cambrian. The Mesozoic Era introduced ever more effective predators with increased encephalization. The modern expansion was the last major increase in biodiversity. This Modern Period has persisted, survived extinction events, and possibly expanded ever since. Was evolution to these forms inevitable because there is a basic advantage to a multicellular eukaryotes (together with their attached and dependent microbial communities) by their maintaining an enormous diversity of metabolic activities and behaviors? Is the microbial world actually dominated whenever large three-dimensional beings develop the physiological and homeostatic mechanisms to exist? Did the microbial world reflect these major changes in evolution or has there been essentially nothing new since the Proterozoic except for some new enzymes that allowed utilization of and protection from the toxic effects of oxygen?

The widespread distribution of specific genes for biodegradation found in microorganisms isolated from each of four continental regions of Mediterranean sclerophyllous ecosystems (California, Chile, South Africa, and Western Australia) and two boreal forests (Russia and Canada) showed no common strains between the continents but with ARDRA (a restriction fragment length polymorphism of PCR amplified rRNA fragments) researchers found that about a quarter of the strains from different continents showed identical bands (relatedness)

to strains from other continents (Tiedje et al. 1994). Possibly the distribution of these specific genes was initiated in Pangea and persisted through the break-up of Gondwanaland.

Are there any new microbes?
The U.S. Dept of Energy program on the Origins of Deep Subsurface Microbes is finding microbes which apparently have existed in a quiescent state for millions of years in subsurface rocks. Work with tracers has indicated they are clearly not contaminants from the sample recovery processes. Comparison of DNA and amino acid sequences of specific enzymes from these organisms for conserved regions with microbes showing recent and active growth should be most enlightening. Differences in functional process enzymes and essential housekeeping enzymes should be especially interesting, as these are supposedly not subjected to the same selection pressures. The diversity of the third or wobble base in the codons for conserved parts of these macromolecules could be very interesting although the interpretation for the control "modern" organism is a major problem – how long since it was released from a subsurface quiescent status? A truly exciting control could be found in chemical signatures from subsurface microbes if they were actually found in the vestigial flood plains of Mars!

Conclusions

With new methods such as the SLB/DNA analysis, the soil microbiota can be defined. Questions involving this most complex microbial ecosystem with the most diverse biota and versatile biochemistry, can now be subjected to quantitative analysis. It may be possible to define soil "quality" based on the viable biomass, community structure and nutritional/physiological status of the soil microbiota and make valuable predictions of crop yields or bioremediation effectiveness. These methods, with their roots in Chemical Ecology, represent detailed application of chemistry and often require sophisticated analytical technology by utterly reductionist chemists. The interpretation, however, with its absence of the familiar landmarks of morphology indicating function and taxonomic relationships, requires a more holistic view of the ecosystem than is required in much of macroecology. The holistic analysis that is proving essential in microbial ecology may benefit macroecology. Per Brinck anticipated the power of Chemical Ecology and brought it into Ecology. The students, and those of us privileged to have been visiting scientists at this great Ecology Center at Lund University, now have the opportunity to lead the way.

Acknowledgements – Research in this laboratory is currently supported by the U.S. Dept of Defense, Office of Naval Research; U.S. Dept of Energy, Subsurface Science Program and programs through Martin Marietta Energy Systems; National Water Research Inst. The research work reported here from our laboratory is the result of the dedication of many colleagues but the incomparable laboratory skills and insight into its application by David B. Ringelberg have made signature lipid biomarker analysis a powerful tool.

References

Aldhous, P. 1994. Biodiversity: Ecologists draft plan to dig in the dirt. – Science 264: 1521.

Angell, P., Arrage, A., Mittelman, M. W. and White, D. C. 1993. On-line, non-destructive biomass determination of bacterial biofilms by fluorometry. – J. Microb. Meth. 18: 317–327.

Arrage, A. A., Vasishtha, N., Sunberg, D., Baush, G., Vincent, H. L. and White, D. C. 1995. On-line monitoring of biofilm biomass and activity on antifouling and fouling-release surface using bioluminescence and fluorescence measurements during laminar-flow. – J. Indust. Microbiol., in press.

Baird, B. H., Nivens, D. E., Parker, J. H. and White, D. C. 1985. The biomass, community structure and spatial distribution of the sedimentary microbiota from a high-energy area of the deep sea. – Deep Sea Res. 32: 1089–1099.

Balkwill, D. L., Leach, F. R., Wilson, J. T., McNabb, J. F. and White, D. C. 1988. Equivalence of microbial biomass measures based on membrane lipid and cell wall components, adenosine triphosphate, and direct counts in subsurface sediments. – Microb. Ecol. 16: 73–84.

Bergh, Ö., Börsheim, K. Y., Bratbak, G. and Mikal, H. 1989. High abundence of viruses found in aquatic environments. – Nature 340: 467–468.

Chalfie, M., Tu, Y., Euskirchen, G., Ward, W. W. and Prasher D. P. 1994. Green fluorescent protein as a marker for gene expression. – Science 263: 802–805.

Dowling, N. J. E., Widdel, F. and White, D. C. 1986. Phospholipid ester-linked fatty acid biomarkers of acetate-oxidizing sulfate reducers and other sulfide forming bacteria. – J. Gen. Microbiol. 132: 1815–1825.

Edlund, A., Nichols, P. D., Roffey, R. and White, D. C. 1985. Extractable and lipopolysaccharide fatty acid and hydroxy acid profiles from *Desulfovibrio* species. – J. Lipid Res. 26: 982–988.

Findlay, R. H. and White, D. C. 1983. Polymeric beta-hydroxyalkanoates from environmental samples and *Bacillus megaterium*. – Appl. Environ. Microbiol. 45: 71–78.

– , Trexler, M. B., Guckert, J. B. and White, D. C. 1990a. Laboratory study of disturbance in marine sediments: response of a microbial community. – Mar. Ecol. Prog. Ser. 61: 121–133.

– , Trexler, M. B. and White, D. C. 1990b. Response of a benthic microbial community to biotic disturbance. – Mar. Ecol. Prog. Ser. 61: 135–148.

Gehron, M. J. and White, D. C. 1982. Quantitative determination of the nutritional status of detrital microbiota and the grazing fauna by triglyceride glycerol analysis. – J. Exp. Mar. Biol. 64: 145–158.

Guckert, J. B., Hood, M. A. and White, D. C. 1986. Phospholipid, ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: increases in the *trans/cis* ratio and proportions of cyclopropyl fatty acids. – Appl. Environ. Microbiol. 52: 794–801.

– , Ringelberg, D. B., White, D. C., Henson, R. S. and Bratina, B. J. 1991a. Membrane fatty acids as phenotypic markers in the polyphasic taxonomy of methylotrophs within the proteobacteria. – J. Gen. Microbiol. 137: 2631–2641.

– , Nold, S. C., Boston, H. L. and White, D. C. 1991b. Periphyton response along an industrial effluent gradient: Lipid-based physiological stress analysis and pattern recognition of microbial community structure. – Can. J. Fish. Aquat. Sci. 49: 2579–2587.

Heipieper, H.-J., Diffenbach, R. and Keweloh, H. 1992. Con-

version of *cis* unsaturated fatty acids to *trans*, a possible mechanism for the protection of phenol degrading *Pseudomonas putida* P8 from substrate toxicity. – Appl. Environ. Microbiol 58: 1847–1852.
Heitzer, A., Malachowsky, K., Tonnard, J. E., Bienkowski, P. R., White, D. C. and Sayler, G. S. 1994. Optical biosensor for environmental on-line monitoring of naphthalene and salicylate bioavailability using an immobilized bioluminescent catabolic reporter bacterium. – Appl. Environ. Microbiol. 60: 1487–1494.
Kehrmeyer, S. R., Appelgate, B. M., Pinkert, H. C., Hedrick, D. B., White, D. C. and Sayler, G. S. 1994. Combined lipid/DNA extraction method for environmental samples. – J. Microbiol. Methods, in press.
Kohring, L. L., Ringelberg, D. B., Devereux, R., Stahl, D. A., Mittelman, M. W. and White, D. C. 1994. Comparison of phylogenetic relationships based on phospholipid fatty acid profiles and ribosomal RNA sequence similarities among dissimilatory sulfate-reducing bacteria. – FEMS Microbiol. Lett. 119: 303–308.
Lennarz, W. J. 1970. Bacterial lipids. – In: Wakil, S. (ed.), Lipid metabolism. Academic Press, New York, pp. 155–183.
Minnikin, D. E. and Abdolrahimzadeh, H. 1974. The replacement of phosphatidylethanolamine and acidic phospholipids by ornithine-amide lipid and a minor phosphorus-free lipid in *Pseudomonas fluorescens* NCMB129. – FEBS Lett. 43: 257–260.
Moriarty, D. J. W., Boon, P. I., Hanson, J. A., Hunt, W. G., Poiner, I. R., Pollard, P. C., Skyring, G. W. and White, D. C. 1985. Microbial biomass and productivity in seagrass beds. – Geomicrobiol. J. 4: 21–51.
Napolitano, G. E., Hill, W. R., Guckert, J. B., Stewart, J. B., Nold, S. C. and White, D. C. 1994. Changes in periphyton fatty acid composition in chlorine-polluted streams. – J. N. Am. Benthol. Soc. 13: 237–249.
Nichols, P. D., Palmisano, A. C., Rayner, M. S., Smith, G. A. and White, D. C. 1990. Occurrence of novel c_{30} sterols in Antarctic sea-ice diatom communities during a spring bloom. – Organic Geochem. 15: 503–508.
Nickels, J. S., King, J. D. and White, D. C. 1979. Poly-beta-hydroxybutyrate accumulation as a measure of unbalanced growth of the estuarine detrital microbiota. – Appl. Environ. Microbiol. 37: 459–465.
Ogram, A., Sayler, G. S. and Barkay, T. 1987. The extraction and purification of microbial DNA from sediments. – J. Microbial Meth. 7: 57–66.
Parker, J. H., Smith, G. A., Fredrickson, H. L., Vestal, J. R. and White, D. C. 1982. Sensitive assay, based on hydroxy-fatty acids from lipopolysaccharide lipid A for gram negative bacteria in sediments. – Appl. Environ. Microbiol. 44: 1170–1177.
Phelps, T. J., Murphy, E. M., Pfiffner, S. M. and White, D. C. 1994. Comparison of geochemical and biological estimates of subsurface microbial activities. – Microbial Ecol. 28: 335–349.
Rice, J. F., Fowler, R. F., Arrage, A. A., White, D. C. and Sayler, G. S. 1995. Effects of external stimuli on environmental bacterial strains harboring an *algD-lux* bioluminescent reporter plasmid for studies of corrosive biofilms. – J. Indust. Microbiol. In press.
Ringelberg, D. B., Stair, J. O., Norby, R. J., O'Neill, E. G., Zac, D. R. and White, D. C. 1995. Elevated atmospheric CO_2 and the below-ground microbiota of *Quercus alba* L. – Global Biol. 2: in review.
Smith, G. A., Nichols, P. D. and White, D. C. 1986. Fatty acid composition and microbial activity of benthic marine sediments from McMurdo Sound, Antarctica. – FEMS Microbiol. Ecol. 32: 219–231.
– , Davis, J. D., Muscat, A., Moe, R. and White, D. C. 1989. Lipid composition and metabolic activities of benthic nearshore microbial communities of Arthur Harbor, Antarctic Peninsula: Comparisons with McMurdo Sound. – Polar Biol. 9: 517–524.
Tiedje, J. M., Fulthorpe, R. R., Rhoades, A. and Bezbodnikov, S. 1994. Molecular biogeography: how uniform is microbial dispersal? – Mol. Ecol. 3: 620.
Torsvik, V., Goksøyr, J., Daae, F. L., Sørheim, R., Michalsen, J. and Salte, K. 1994. Use of DNA analysis to determine the diversity of microbial communities. – In: Ritz, K., Dighton, J. and Giller, K. E. (eds), Beyond the biomass, compositional and functional analysis of soil microbial communities. Wiley, New York, pp. 39–48.
Tunlid, A. and White, D. C. 1991. Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of the microbial communities in soil. – In: Bollag, J.-M. and Stotzky, G. (eds), Soil biochemistry. Marcel Dekker, New York, pp. 229–262.
– , Baird, B. H., Trexler, M. B., Olsson, S., Findlay, R. H., Odham, G. and White, D. C. 1985. Determination of phospholipid ester-linked fatty acids and poly beta hydroxybutyrate for the estimation of bacterial biomass and activity in the rhizosphere of the rape plant *Brassica napus* (L.). – Can. J. Microbiol. 31: 1113–1119.
Walker, J. T., Sonesson, A., Keevil, C. W. and White, D. C. 1993. Detection of *Legionella pneumophila* in biofilms containing a complex microbial consortium by gas chromatography-mass spectrometric analysis of genus-specific hydroxy fatty acids. – FEMS Microbiol. Lett. 113: 139–144.
Ward, D. M., Bateson, M. M., Weller, R. and Ruff-Roberts, A.-L. 1992. Ribosomal RNA analysis of microorganisms as they occur in nature. – Adv. Microbial Ecol. 12: 219–286.
Welch, D. F. 1991. Applications of cellular fatty acid analysis. – Clinical Microbiol. Rev. 4: 422–438.
White, D. C. 1988. Validation of quantitative analysis for microbial biomass, community structure, and metabolic activity. – Adv. Limnol. 31: 1–18.
– 1994. Is there anything else you need to understand about the microbiota that cannot be derived from analysis of nucleic acids? – Microb. Ecol. 28: 163–166.
– , Davis, W. M., Nickels, J. S., King, J. D. and Bobbie, R. J. 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. – Oecologia 40: 51–62.
– , Bobbie, R. J., Nickels, J. S., Fazio, S. D. and Davis, W. M. 1980. Nonselective biochemical methods for the determination of fungal mass and community structure in estuarine detritial microflora. – Bot. Mar. 23: 239–250.
– , Ringelberg, D. B., Guckert, J. B. and Phelps, T. J. 1991. Biochemical markers for in situ microbial community structure. – In: Fliermans, C. B. and Hazen, T. C. (eds), Proceedings of the First International Symposium on Microbiology of the Deep Subsurface. WSRC Information Service, Aiken, SC. pp. 4–45 to 4–56.
Wilson, E. O. 1994. Naturlist. – Island Press, Washington.