Molecular characterization of microbial populations in the water column of a stratified lake (Paul Lake, Michigan) related to chemical zonation

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RNA extracted from water samples collected in Paul Lake (Michigan) was probed with radiolabeled oligonucleotides complementary to the small-subunit ribosomal RNA (SSU rRNA) of all three domains of life (Eucarya, Bacteria, and Archaea), each domain individually, and specific groups of bacteria and archaea (sulphate-reducing bacteria (SRBs), 'cold-water' crenarchaeota). Probe hybridization provides an approximation of metabolic activity because ribosome production is growth-rate dependent, although the details of growth-rate regulation differ among prokaryotic species. When our probe hybridization profiles were correlated with the physical and chemical stratification of Paul Lake, both expected and unexpected population distributions were found.

Site description

Paul Lake is located in UNDERC (University of Notre Dame Environmental Research Center), at the border of Wisconsin and the Upper Penninsula of Michigan. It is a mesotrophic brown-water kettle lake surrounded on three sides by moraine ridges. Water enters Paul Lake by precipitation and seepage. Its protected location and high ratio of depth to surface area help maintain stratification, although mixing is possible in autumn.

The concentration of soluble reactive phosphorus in the surface water is very low, but nutrient regeneration at the oxic/anoxic transition promotes phytoplankton blooms just above this interface. The fish community is dominated by largemouth bass (*Micropterus salmoides*), and zooplankton by large-bodied *Daphnia pulex*, *Daphnia rosea*, and *Diaptomus oregonensis*. Little is known about the microbial community. Blooms of photosynthetic sulphur bacteria have been found below the oxycline, peaking at approximately 6.5 m (Parkin and Brock, 1980). Manganese-encrusted bacteria were observed just above the oxycline, suggesting the possibility of

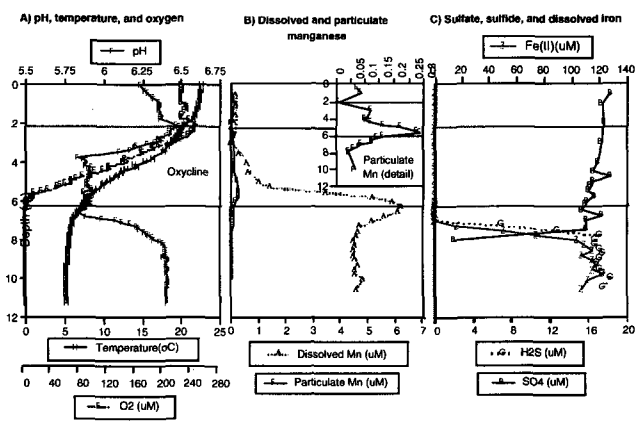

FIG. 1. Physical and chemical characterization.

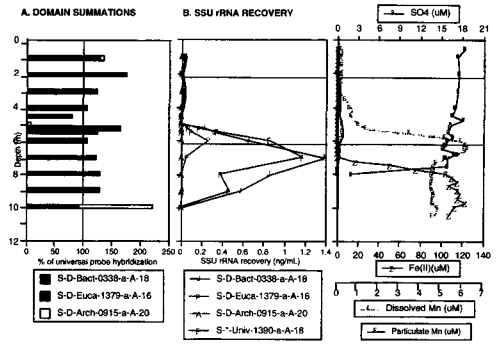

FIG. 2. Hybridization to universal and domain probes.

bacterially-mediated manganese oxidation (Lienemann *et al.*, 1997).

Results

Oligonucleotide probes targeting small-subunit ribosomal RNA (SSU rRNA) were used to study the distribution of microbial populations with depth. At the time of sampling (August 8, 1996), oxygen concentration decreased from 250 μM at 2 m to below the detection limit at 6 m (Fig. 1).

SSU rRNA recovery was highest near the top of the anoxic hypolimnion (Fig. 2). Hybridization to a universal probe and to probes targeting the bacterial and eukaryotic domains was low above 5 m, and near zero at 5 m. Eukaryotic probe hybridization peaked at 6 m. Bacterial probe hybridization peaked at 8 m, then declined to near detection limits at 10 m. No samples were taken between 10 m and the bottom of the lake at 12 m.

Archaeal probe (D-Arch-0915-a-A-20) hybridization peaked at the shallowest depth sampled (0.5 m) and again at 9 m (Fig. 3A). Increasing hybridization with depth below the oxycline is expected, as archaeal methanogens are often found in the anaerobic zones of freshwater lakes. Archaeal probe hybridization in near-surface samples is unlikely to be due to methanogens, as the known species are obligate anaerobes, although anaerobic niches might be found on particles or within small eukaryotes.

The archaeal probe hybridization peak in the epilimnion can be accounted for partly by 'cold-water' crenarchaeota, an archaeal group found in other aerobic environments (Fig. 3B). Two crenarchaeotal probes (S-K-Cren-0667-a-A-15 and S-K-Cren-0554-a-A-*) designed to target the same known crenarchaeotal SSU rRNA sequences showed different hybridization patterns, implying that the crenarchaeota may be more diverse than is currently realized. At 4m, hybridization to the two probes was approximately equal.

Two probes targeting the delta proteobacteria were used (Fig. 3C). S-F-Dsv-0687-a-A-16 targets the *Desulfovibrio* group and some of the *Geobacter* group, while S-*-Dsb-0804-a-A-18 targets *Desulfobulbus* and relatives. As expected, hybridization to both probes peaked in the region of sulphate and metal reduction, at 7m. However, there is also a smaller peak of S-*-Dsb-0804-a-A-18 hybridization at 4m, where oxygen concetration was approximately 140 μM.

Although once thought to be obligate anaerobes, SRB are increasingly being found in oxic environments. SRB have been isolated from oxic lake sediments (Sass *et al.* 1997), and sulphate reduction has been measured in the oxic zones of microbial mats (Canfield and Des Marais, 1991) and coastal marine sediments (Moeslund *et al.*, 1994). Of particular relevance to the present study, Watras *et al.* (1995) found two peaks of sulphate reduction activity in Pallette Lake, Wisconsin—a small peak just above the oxic/anoxic transition, and a larger peak just below it.

S-*-Dsb-0804-a-A-18 hybridization represented a large fraction (up to 50%) of universal probe hybridization between 2 and 5 m. Whether these organisms are exposed to oxygen or living in anaerobic niches remains to be shown; in either case, their abundance argues an important role.

We believe this study demonstrates the usefulness of molecular methods for describing microbial populations in lakes. They may be particularly useful in the study of diel and seasonal changes in fine-scale microbial population structure, if specific probes for the groups of interest can be designed.

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