Monitoring Bacterial Genetic Diversity in a Freshwater Lake Using Temporal Temperature Gradient Gel Electrophoresis and DNA Sequence Analysis

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Introduction

Microbial ecology is now firmly focused on the genotypic analysis of naturally occurring assemblages, facilitated by the development of molecular biological techniques that enable amplification and sequencing of DNA extracted directly from the environment. The ubiquitous prokaryotic 16S ribosomal RNA (rRNA) gene has been the most widely targeted molecule for these studies because regions of nucleotide sequence conservation allow polymerase chain reaction (PCR) amplification to generate DNA fragments incorporating the variable sequence regions from which phylogeny can be inferred. In this paper, we describe the use of temporal temperature gradient gel electrophoresis (TTGE) as the method of choice for sequence variation studies. In the commonly used denaturing gradient gel electrophoresis (DGGE) (Fischer and Lerman 1979), electrophoretic separation is performed at high temperature in the presence of a chemical gradient. In TTGE, the denaturing environment is formed by a constant concentration of denaturant in the gel in combination with a gradual increase in temperature over the course of the separation. Thus, by using TTGE, one avoids pouring chemical gradient gels (Zoller et al. 1998).

TTGE is an attractive technique for the molecular microbial ecologist, as it separates similar length PCR products according to sequence variation typical of 16S rRNA genes. PCR products separated by TTGE into discrete bands, in theory, represent individual DNA species from a microbial consortium, so banding profiles provide a rapid insight into genetic diversity in situ. Excision and sequence analysis of gel-resolved DNA further permits phylogenetic interpretation of a microbial community structure that may be correlated with environmental factors.

Ammonia-oxidizing bacteria mediate the nitrification stage of the nitrogen cycle; hence their activity is of ecological importance, but the influence of environmental parameters upon their community structure is largely unknown (Hastings et al. 1998). Described here is the combined application of TTGE and sequence analysis of gel-resolved 16S rDNA PCR products amplified from freshwater *Nitrosospira* spp. collected throughout a 12-month period in an attempt to observe temporal genetic variation within this functionally important microbial group.

Methods

Genomic DNA, extracted from lakewater samples collected at monthly intervals from January to December, was PCR-amplified to generate 240 bp GC-clamped fragments of the 16S rRNA gene spanning the variable V3 region from ammonia-oxidizing bacteria belonging to the genus *Nitrosospira* (Hastings et al. 1998, Hastings et al. 1997). Approximately 200 ng of PCR product was mixed with 2x Bromophenol Blue loading dye and electrophoresed in a 6% polyacrylamide gel (acrylamide/bis 37.5:1) containing 7 M urea, 20% formamide, 2% glycerol in 1.25x TAE buffer (50 mM Tris-Cl, 25 mM acetic acid, 1.25 mM EDTA, pH 8.0) using the DCode™ universal mutation detection system at 100 V for 16 hr across a temperature range of 40–55°C and a temperature ramp of 1°C/hr. After electrophoresis, gels were equilibrated in 1.25x TAE buffer for 15 min, stained in ethidium bromide (25 mg/ml in TAE) for 20 min, and washed in TAE before viewing and photographing under ultraviolet (UV) light (302 nm). Various individual DNA bands were cut from the gel with a sterile scalpel, eluted by passive diffusion into 1.25x TAE, and sequenced.

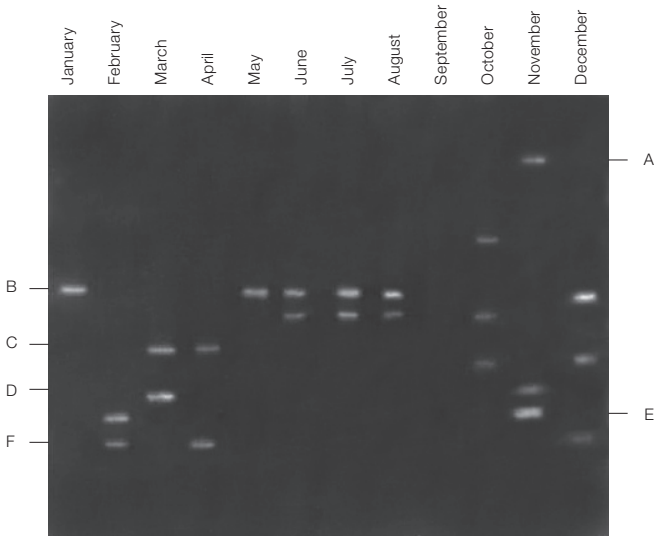

Fig 1. TTGE gel of *Nitrosospira* 16S rDNA PCR products amplified from monthly freshwater samples. DNA bands representing positions A–F were excised and sequenced for the determination of phylogenetic relatedness.

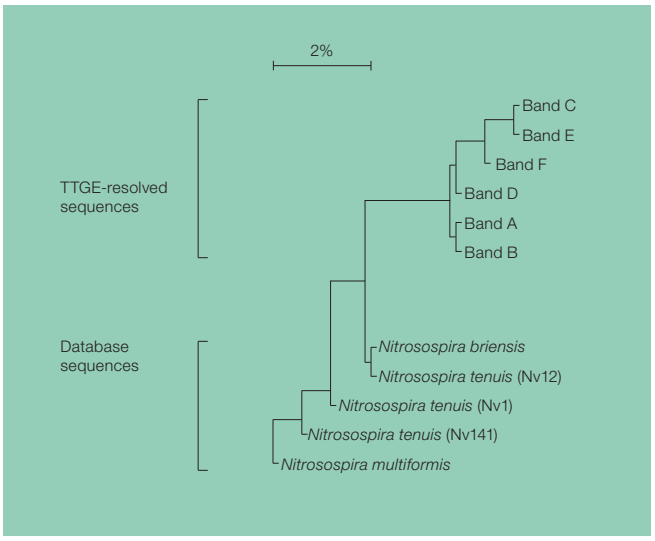

Fig. 2. Dendrogram indicating phylogenetic relationships among 16S rDNA PCR products amplified from freshwater *Nitrosospira* resolved by TTGE. Scale bar represents 2% difference in nucleotide sequence.

Results and Discussion

TTGE analysis of freshwater *Nitrosospira* 16S rDNA PCR products revealed both genotypic continuity and variation between individual samples (Figure 1). Comigrating DNA bands (position B) were observed during the periods of lakewater overturn and stratification, suggesting the presence of a predominant *Nitrosospira* genotype during these times. Spring and autumn samples, corresponding to formation and breakdown of a stratified water column respectively, demonstrated the predominance of other strains of *Nitrosospira* by the observation of differentially migrating PCR products (positions A, C, D, E, and F). While myriad other DNA bands present throughout the gel indicated an overall complex genetic profile within this *Nitrosospira* group, the relative quantity of DNA in individual bands suggested the numerical dominance by a number of strains (positions A–F) whose prevalence appeared to be temporally variable. Six DNA bands (positions A–F) were excised from the gel and sequenced. Phylogenetic analysis of these sequences grouped them as closely related genotypes, but in a separate cluster were grouped to previously recognized *Nitrosospira* spp. (Head et al. 1993).

Confidence in dendrogram topology is limited due to the restricted quantity of sequence data available for scrutiny (~200 bp), yet the acute resolving power of the TTGE technique enabled the separation of *Nitrosospira* 16S rRNA genes differing by as little as one base pair across the fragment examined.

TTGE is a reliable technique and a valuable tool for microbial diversity studies. By loading PCR products in adjacent lanes, we were able to compare band profiles and, hence, genetic diversity from different environmental samples. Additionally, sequencing the gel-resolved DNA enables phylogenetic analysis of PCR products, eliminating the need to clone DNA fragments and, thus, simplifying our analysis.

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