Sample introduction:

Denaturing gradient gel electrophoresis (DGGE) is a popular method for assessing the structure of microbial communities in environmental samples (Muyzer and Smalla, 1998). The technique is based on the electrophoretic separation of PCR-generated double stranded DNA in an acrylamide gel containing a gradient of a denaturant. As the DNA encounters an appropriate denaturant concentration, a sequence-dependent partial separation of the double strands occurs. This conformational change in the DNA tertiary structure causes a reduced migration rate and results in a DNA band pattern representative of the sampled microbial community. Modern image analysis systems have proven to be of value for the analysis of DGGE bands and their associated patterns. For instance, pairwise matching of DGGE bands in separate gel lanes has facilitated the calculation of similarity coefficients to describe relationships between communities (van der Gucht et al., 2001; Sigler et al., 2002). Additionally, the use of common diversity indices that incorporate band number and intensity as surrogates for phylotype number (Casamayor et al., 2000) and abundance (Øvreås et al., 1997; Konopka et al., 1999; Nübel et al., 1999; McCaig et al., 2001; Sigler

and Zeyer, 2002a), respectively, is also popular. However, this application of band information is often limited to less complex systems due to PCR amplification biases including preferential- and nonspecific amplification (reviewed by van Wintzingerode et al., 1997; Suzuki and Giovannoni, 1996) and heterogeneity in rrn copy number (Farrelly et al., 1995). Regardless of the methods chosen to interpret banding patterns, key to the success of DGGE-based community structure analysis is the separation of PCR products that results in the optimum resolution of as many potential phylogenetic markers as possible.

As with many molecular methods, the steps involved in DGGE analysis are more or less consistent among differing laboratories, but not standardized. In general, the PCR product length analyzed is between 200 and 600 base pairs (bp). The acrylamide percentage of the gel is commonly either 6 or 8 percent and most runs are performed at a temperature of 60^{o} C across denaturant concentrations from as low as 20% to as high as 70% or more (a 100% denaturing solution is defined as 40% [vol/vol] formamide and 7 M urea). However, much inconsistency exists in the choice of electrophoresis volt-hours (V·h), which is a function of applied voltage and running time. This inconsistency is reflected in the applied V·h described throughout the DGGE

literature, which ranges from a minimum of 130 V for 3.5 h (455 V·h; Cocolin et al., 2001) to 2100 V·h (100 V for 21 h; Gejman et al., 1998). Since our preliminary experiments have shown that extended electrophoresis times resulted in sub-optimal band separation and resolution, this study was conducted to further explore the impact of extended electrophoresis times on community analyses.