Denaturing Gradient Gel Electrophoresis of Natural Virus Samples on the DCode™ System

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Introduction

Denaturing gradient gel electrophoresis (DGGE) is a method of separating DNA fragments of the same size that differ in sequence. In DGGE, polyacrylamide gels are cast with linear gradients of denaturants. A 100% denaturing gel solution is defined as having 40% (v/v) formamide and 7 M urea. DNA fragments of the same size that differ in sequence will not resolve in agarose gels, but will migrate to different positions in a DGGE gel as the DNA denatures.

DGGE makes use of the fact that the melting behavior of double-stranded DNA (dsDNA) is affected by temperature, concentration of denaturant (e.g., urea and/or formamide), and interactions of adjacent base pairs stacked in a helix (Myers et al. 1987). Therefore, discrete segments or domains within a dsDNA molecule denature under different conditions, according to their base composition. As a result of the formation of branched DNA molecules, partial denaturation reduces electrophoretic mobility. Resolution of dsDNA samples of the same length, but differing in sequence, occurs by running the DNA in a polyacrylamide gel that has a continuously increasing concentration of denaturant. Because DNA strands that differ in sequence partially melt at different concentrations of denaturant, their migration slows at different positions in the gel.

In this experiment, we show that parallel DGGE analysis on the DCode universal mutation detection system can be used to analyze sequence differences among virus genes amplified from related viruses and from natural virus communities. Furthermore, we found that a GC clamp on polymerase chain reaction (PCR) primers used for amplification was not required for DGGE with these products. This approach can be used to genetically fingerprint natural virus communities.

Methods

DNA polymerase gene fragments were amplified from a combined template consisting of DNA from cultured viruses (MpV-SP1, MpV-PB8, and CVA-1) belonging to the family Phycodnaviridae. Template DNA was also obtained from natural marine virus samples collected from two inlets on the west coast of Vancouver Island, Canada. Target sequences from all templates were amplified by PCR using degenerate primers that are specific for members of the virus family Phycodnaviridae (Chen and Suttle 1995). We did not need GC clamps to amplify these longer PCR products. Reactions conducted on the combined template and the two natural virus samples all produced an end product of approximately 700 base pairs.

DGGE was conducted using a 6% acrylamide/bis (37.5:1) gel with a parallel denaturing gradient range of 0–60% in 1x TAE (40 mM Tris, 20 mM acetic acid, 1 mM EDTA). The gradient gel was cast using the Bio-Rad Model 475 gradient delivery system. Approximately 300 ng of the cultured virus product was mixed with 12 µl of 2x loading dye (70% glycerol, 0.05% Bromophenol Blue, 0.05% Xylene Cyanol, 2 mM EDTA), while 1 µg of PCR products from the natural communities was combined with 20 µl of 2x loading dye. Electrophoresis was conducted using the DCode system at 135 V for 6 hr at a buffer temperature of 58°C. After electrophoresis, the gel was stained in a 1:10,000 dilution of SYBR Green I (Molecular Probes, Inc.) in 1x TAE buffer overnight. The gel was imaged under ultraviolet (UV) transillumination.

Results and Discussion

Figure 1 shows virus DNA polymerase gene fragments run on a parallel DGGE gel. Lane 1 reveals that PCR amplification from combined templates produced three fragments that correspond to the individual viruses. It has been previously demonstrated that when amplified individually, each of the three viruses produces a unique DGGE band (Short and Suttle 2000). Lanes 2 and 3 reveal unique banding patterns for each virus sample, suggesting that virus community composition is spatially variable. In a related study, DGGE of natural samples revealed both spatial and temporal differences in the genetic composition of natural virus samples (Short and Suttle 1999). Notably, when parallel DGGE-separated mixtures of similar PCR products (Figure 1) were run on a standard agarose gel, they migrated as a single band. Thus, the ability of parallel DGGE to resolve similar-sized fragments produced in a single PCR reaction makes it possible to detect unique sequences in samples of varying genetic diversity. Our data indicate that DGGE can be used to distinguish DNA polymerase gene fragments of the same size from related yet genetically distinct viruses. Furthermore, DGGE distinguishes natural virus samples obtained from different marine locations.

DGGE is a reliable technique to screen the genetic diversity of aquatic virus samples using the DCode system. Reproducible gradients are easily cast with the Bio-Rad gradient delivery system, and buffer temperature can be held constant by the DCode system, ensuring reproducible electrophoresis conditions.

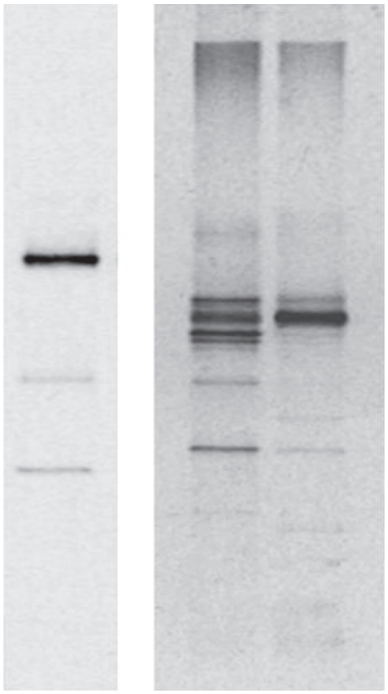

Fig. 1. Parallel DGGE separation of virus DNA polymerase gene fragments run on the DCode system. Lane 1, PCR products amplified from the combined templates of MpV-SP1, MpV-PB8, and CVA-1 DNA; lane 2, PCR products amplified from a virus sample from Grappler Inlet, British Columbia, Canada; lane 3, PCR products amplified from a virus sample from Bamfield Inlet, British Columbia, Canada.

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