P2: 5'-CGCCGCGCCGCCCCGCGCCG
GCCGCGCGCCCCGCCCCGACGGCGG
GTGTAC-3'.

The specificity of this primer is imparted by the underlined region. Theoretically, these primers should amplify a 323-bp section of the 16S rRNA genes of members of the domain Bacteria, including the highly variable V9 region. PCRs were performed by using cloned Pfu DNA polymerase according to the instructions provided by the manufacturer (Promega). PCR products were examined on ethidium bromide-stained agarose gels, and the reaction mixtures yielding products of the expected size were used for DGGE analysis. The temperature cycle for the PCR was 1 min of denaturation at 94°C, 1 min of annealing (see below), and 3 min of primer extension at 72°C. An initial denaturation step in which the temperature of the PCR mixture (without the polymerase) was raised to 94°C for 5 min and then lowered to 80°C for 1 min, at which time the polymerase was added, was used. During an initial touchdown cycle, the annealing temperature was lowered from 53 to 43°C in

and run with 0.5x TAE buffer (13 TAE is 0.04 M Tris base, 0.02 M sodium acetate, and 1.0 mM EDTA; pH adjusted to 7.4). An Ingeny Phor U gel electrophoresis unit was used with glass plates (16 by 18 cm), 1.0-mm spacers, and 1-cm-wide loading wells. A 15-liter aquarium served as the lower buffer chamber. DGGE gels contained a 20 to 60% gradient of urea and formamide (UF) solution increasing in the direction of electrophoresis. A 100% UF solution is defined as 40% (vol/vol) formamide plus 7.0 M urea (1, 20). DGGE was conducted at 60°C, firstly at 20 V for 10 min and then at a constant voltage of 200 V for 5 h.

Silver Staining. Gel was stained with silver staining method (Bassam, B.J. et al., 1991). Firstly, gel was fixed using 10% acetic acid for 2x10 minutes and then rinsed with distilled water for 6 minutes. Subsequently gel was stained for 30 min in 100 ml silver nitrate solution containing 0.1% AgNO₃ and 150 μl formaldehyde/HCHO 37%. After that, gel was rinsed for about 10 seconds in distilled water and immediately soaked with the cold (4-10°C) developing solution (100 ml solution