



Note

PCR-based community structure studies of Bacteria associated with eukaryotic organisms: A simple PCR strategy to avoid co-amplification of eukaryotic DNA

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ABSTRACT

PCR primers targeting conserved regions of the SSU rRNA gene are commonly used in bacterial community studies. For microbes associated with eukaryotes, co-amplification of eukaryotic DNA may preclude the analysis. We present a simple and efficient PCR strategy to obtain pure bacterial rDNA amplicons from samples predominated by eukaryotic DNA.

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During the past two decades, culture-independent methods have become commonly applied for studying the composition of Bacteria in samples (Cardenas and Tiedje, 2008; Tringe and Hugenholtz, 2008). Examples of such methods are Denaturing Gradient Gel Electrophoresis (DGGE), Terminal Restriction Fragment Length Polymorphism (T-RFLP), sequencing of SSU ribosomal DNA (rDNA) clone libraries, and more recently barcoded amplicon 454-sequencing. They all involve a step with PCR amplification of extracted DNA. Typically, fragments of the SSU rRNA (small subunit ribosomal RNA) gene are amplified. PCR primers should ideally target most taxa of Bacteria. Further, the resulting amplicons need to be of a length suitable for further analysis, and the DNA sequence of the amplified region should be informative in phylogenetic or diversity analysis. These factors limit the possibilities in primer design.

In the SSU rRNA gene of Bacteria, two conserved regions corresponding to *E. coli* gene nucleotide positions around 320–355 (e.g. primer 338F; 5'-actcctacgggaggcagcag-3') and 505–535 (e.g. primer 518R; 5'-attaccgcggtctgg-3') have been extensively used for generating PCR products for DGGE analysis (Ahn et al., 2006; Klammer et al., 2008; Muyzer et al., 1993). These primers efficiently amplify an approximately 200 bp fragment for most bacterial taxa. However, the primer sequences exhibit similarity to conserved regions in eukaryotic SSU rRNA genes (Fig. 1), with 100% sequence

homology for the 518R primer sequence to a conserved region in the eukaryotic SSU rRNA gene.

When studying composition of Bacteria in or on eukaryotic organisms, a separation of nucleic acids from Bacteria and Eukarya may be impossible, and the nucleic acid extracts to be examined often predominantly consist of eukaryotic DNA. This may cause problems when using conserved SSU rRNA primers, as previously emphasized by Huys et al. (2008).

We used the primers 338F-GC (for sequence, see legend to Fig. 2) and 518R to study the composition of Bacteria associated to cod larvae (*Gadus morhua*; 4–60 days after hatching) by DGGE. Total DNA was extracted using the Blood and Tissue Kit (Qiagen). PCR reactions were run for 35 cycles (95 °C 30 s, 50 °C 30 s, and 72 °C 60 s) with 2 mM MgCl₂, 0.2 mM of each dNTP, 0.3 μM of each primer, and Taq polymerase and reaction buffer from VWR. Assuming that the total DNA extracts from the cod larvae samples were predominated by eukaryotic cod DNA, we included a control where DNA from Bacteria-free cod muscle was used as template. This control PCR reaction efficiently amplified cod DNA and resulted in a distinct PCR product slightly smaller than 200 bp (Fig. 2A). PCR products obtained from 6 individual cod larvae samples were subjected to DGGE analysis together with the amplicon from sterile cod muscle DNA (Fig. 3A). A band corresponding to the cod muscle PCR product appeared to dominate all the cod larvae DGGE profiles. Sequencing (see band in frame of Fig. 3A) revealed that the band represented an approximately 170 bp *Gadus morhua* SSU rDNA fragment, resulting from primer binding to the SSU rDNA regions shown in Fig. 1.

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