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Differential Amplification of rRNA Genes by Polymerase Chain Reaction

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The polymerase chain reaction (PCR) is used widely to recover rRNA genes from naturally occurring communities for analysis of population constituents. We have found that this method can result in differential amplification of different rRNA genes. In particular, rDNAs of extremely thermophilic archaebacteria often cannot be amplified by the usual PCR methods. The addition of 5% (wt/vol) acetamide to a PCR mixture containing both archaebacterial and yeast DNA templates minimized nonspecific annealing of the primers and prevented preferential amplification of the yeast small-subunit rRNA genes.

Advances in molecular biology have complemented classical microbiological techniques in microbial ecology. Molecular phylogenetic approaches, based on cloning and comparative analysis of 16S rRNA genes of natural microbial communities, have alleviated to some extent the limitations associated with the traditional reliance on culture enrichment techniques for analysis of community diversity (6). It is well recognized that only a minor fraction of the constituents of naturally occurring communities can be recovered in pure culture (11). Isolation of rRNA genes for phylogenetic analysis may be less selective and provide a more representative view of a microbial community structure than do classical techniques, but such an approach has other potential limitations. For instance, biases may be introduced during sample collection, and there may be selection of certain DNAs during cloning steps.

Isolation of rRNA genes was greatly facilitated with the advent of the polymerase chain reaction (PCR) (9). In analysis of rDNA from mixed populations of organisms, primers complementary to universally conserved regions of rRNA allow for the amplification of a broad spectrum of rDNA types (4, 10, 12). However, as we document here, there are serious concerns that preferential amplification of some DNA templates may lead to a biased view of the community structure.

We illustrate the potential for preferential amplification of some rRNA genes using a mixture of DNAs purified from two hyperthermophilic archaebacteria strains, GE5 (3) and AL2 (8), and *Saccharomyces cerevisiae*. Small-subunit rDNA was amplified by PCR using a forward primer which corresponds to nucleotide positions 2 to 21 (5′-TTCCGGT TGATCCYGCCGGA-3′) of *Escherichia coli* 16S rRNA and a reverse primer corresponding to the complement of positions 1510 to 1492 (5′-GGTTACCTTGTTACGACTT-3′). These and similar primers are widely used for small-subunit rDNA amplification. Each 100-μl amplification reaction mixture contained DNA (such that equal copy numbers of the small-subunit rRNA genes were present); 10 μl of 10× reaction buffer (100 mM Tris-HCl, pH 8.3; 500 mM KCl; 15 mM $MgCl_2$); 5 μl of 1% Nonidet P-40 (Sigma, St. Louis, Mo.); 1.5 mM (each) dATP, dGTP, dTTP, and dCTP; 0.5 μg of each primer; and 1 U of *Taq* polymerase. All reactions were run in duplicate and overlaid with mineral oil. The reaction mixtures were incubated in a thermal cycler (Perkin-Elmer Cetus) for 4 min at 94°C and then underwent 30 amplification cycles of 1 min 30 s at 92°C, 1 min 30 s at 50°C, and 72°C for 2 min which was extended for 5 s after each cycle. Ten percent of each reaction mixture was resolved by agarose gel electrophoresis. Acetamide (5%, wt/vol) was added to some reactions to investigate whether the preferential amplification could be alleviated by a denaturant (7, 13). Increasing or decreasing the annealing temperature, including an initial 94°C denaturation cycle, and decreasing the elongation time to 30 s did not alter the results obtained (data not shown).

In standard reactions in which both yeast and archaebacterial DNAs were present, the yeast rDNA, distinguishable from archaebacterial rDNA by its different size, was preferentially amplified (Fig. 1, lanes 7, 8, 15, and 16). However, in the presence of acetamide both the archaebacterial and the yeast rDNAs were amplified. Therefore, acetamide alleviates to a considerable extent selective amplification of these rDNAs. The addition of acetamide did not appear to significantly impair the yield of the PCR products.

Addition of 5% acetamide to reactions containing archaebacterial DNA also enhanced the specificity of the reaction. In one case (Fig. 1, lanes 3 and 4), no amplification was obtained in the absence of acetamide and at various concentrations of DNA (data not shown) while amplification did occur in the presence of acetamide. The addition of acetamide to reaction mixtures containing strain AL2 DNA prevented nonspecific priming, indicated by the presence of the 1,550-bp product in identical reactions in which acetamide was absent (Fig. 1, lanes 11 and 12). Similar suppression of multiple amplification products, presumably due to nonspecific priming, has been observed by using acetamide in amplification of eubacterial DNAs (data not shown). There are other approaches to facilitate denaturation in PCRs, for instance, alkali denaturation (1), "hot starts" (2), and inclusion of cosolvents (5). We have not extensively explored the utility of these in alleviating template bias during PCR.

These results have significant implications for the characterization of community diversity by analysis of rRNA genes amplified by PCR. Although this strategy offers a relatively

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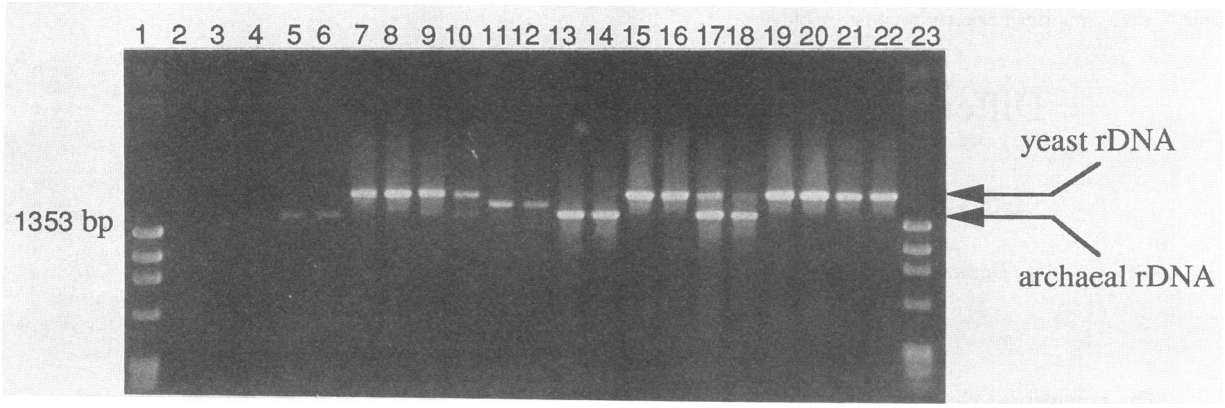

FIG. 1. Differential amplification of yeast and archaebacterial small-subunit rDNA. Ten percent of the amplification products described in the text was resolved in a 1.2% agarose gel. Lanes 1 and 23, ϕX174/*Hae*III standard; lane 2, PCR without template; lanes 3 and 4, GE5 DNA; lanes 5 and 6, GE5 DNA and 5% acetamide; lanes 7 and 8, GE5 DNA and yeast DNA; lanes 9 and 10, GE5 DNA, yeast DNA, and 5% acetamide; lanes 11 and 12, AL2 DNA; lanes 13 and 14, AL2 DNA and 5% acetamide; lanes 15 and 16, AL2 DNA and yeast DNA; lanes 17 and 18, AL2 DNA, yeast DNA, and 5% acetamide; lanes 19 and 20, yeast DNA; lanes 21 and 22, yeast DNA and 5% acetamide.

rapid means of obtaining information about microbial community composition, it is potentially selective. Selection may be minimized by maximizing the conditions for template denaturation and primer annealing, for instance, with the use of acetamide.

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