Direct Genomic DNA Amplification from Autoclaved Infectious Microorganisms Using PCR Technology

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A simple procedure is presented that will enable one to amplify readily DNA sequences directly from human pathogenic microorganisms with a minimum risk of infection. PCR technology has been used to amplify DNA plasmid inserts from pure *Escherichia coli* culture.⁽¹⁾ Recently, we have extended this methodology to the direct amplification of host genomic DNA sequences from a wide range of microorganisms.⁽²⁾ However, this scheme did not include PCR manipulation of highly infectious microorganisms. In an effort to extend this method in a safe manner, three pathogenetically related *Mycobacteria* were cultured in an appropriate hospital environment in glass universals; upon receipt of these cultures, they were autoclaved (15 lb/inch² for 15 min). Approximately 100 µl of each *Mycobacterium* culture was removed, pelleted by centrifugation, resuspended in 25 µl of water, and overlaid with mineral oil. Proteinase K was then added (50 µg/ml) and the samples were incubated at 55°C for 10 min, following which proteinase K was denatured by incubating the sample at 95°C for 10 min. The components of the PCR were then added and the reaction was carried out for 30 cycles in a final volume of 100 µl. In this PCR we used conserved primers to amplify the V6 variable region of 16S ribosomal gene sequences (Fig. 1).⁽²⁾

We have also used this protocol to amplify similar *Mycobacteria* DNA sequences by physically scraping autoclaved material from slope culture using disposable sterile loops, resuspending the scraped material in an appropriate volume of water, removing an aliquot, lysing as above, and subsequently carrying out the PCR. However, we find direct amplification from autoclaved liquid culture more convenient. In a preliminary analysis as to the length of PCR product that may be amplified after autoclaving, successful amplification of a 1200-bp ribosomal sequence has been observed; i.e., the region corresponding to the 5' of the V2 variable region to the 3' end of the V6 variable region of the *Mycobacterium tuberculosis* 16S ribosomal gene.⁽²⁾ We envisage, however, that because of the shearing effect of autoclaving on DNA, there may be a prob-

FIGURE 1 A 3.5% NuSieve agarose gel of PCR-amplified 110 bp 16S ribosomal V6 variable regions (later confirmed by sequencing) from autoclaved *M. tuberculosis* (lane 1), *M. avium* (lane 2), and *M. bovis* (lane 3), and pBR322 *Hae*III size markers (lane 4).

lem in amplifying longer sequences of DNA in some instances.

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