The 16s/23s Ribosomal Spacer Region as a Target for DNA Probes to Identify Eubacteria

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Variable regions of the 16s ribosomal RNA have been frequently used as the target for DNA probes to identify microorganisms. In some situations, however, there is very little sequence variation observed between the 16s rRNA genes of closely related microorganisms. This study presents a general method to obtain species-specific probes using the spacer (intergenic) region between the 16s and 23s rRNA genes. The overall strategy is analogous to that which has previously been developed for the variable regions of the 16s rRNA genes. Sequence analysis of the 16s rRNA and 23s rRNA coding sequences flanking the spacer regions resulted in the design of PCR primers that can be used to amplify the spacer regions of a wide range of eubacteria. Sequencing the amplified spacer region then gives rise to the information that can be used to select specific DNA sequences for use as a DNA probe or for the generation of specific PCR primers to a microorganism of interest. In this study the approach to develop specific DNA markers for members of the genus *Clostridium* is described in detail. A specific DNA oligonucleotide probe and PCR primers have been designed for *Clostridium perfringens* that distinguish it from other organisms in the genus.

The application of DNA probe methodology to the identification and detection of microorganisms is becoming well established in the field of diagnostic bacteriology. Fundamental to such a technology is the ability to define suitable nucleic acid sequences that identify a particular microorganism or group of related microorganisms. DNA probes have been used successfully in the identification and detection of microorganisms.[1,2] In general, nucleic acid sequences that are used as DNA probe targets for microorganisms fall into five main categories: (1) DNA sequences that code for antigens, (2) DNA sequences that code for toxins, (3) DNA sequences identified by differential hybridization using total DNA probes from related species against a DNA bank made from the microorganism of interest, (4) unique plasmid-borne DNA sequences, and (5) ribosomal RNA (rRNA) sequences.

In the case of the first four categories, the means by which the DNA targets are selected involve laborious cloning methodologies and frequently a signficant amount of biochemical or immunological data. Subsequently, cross-reactivity, nonspecificity, and the possibility of the loss of the target sequences due to recombination and deletion events, especially with relation to plasmid-borne sequences, results, in some cases, in a limited usefulness of these probes. rRNAs in general have been the main targets for the generation of DNA markers for microorganisms, and we have used this region as the target for DNA probes for a number of microorganisms.[2] The major disadvantage these sequences have as candidates for DNA markers is that the "variable" regions can almost be identical when closely related microorganisms are examined, resulting in the need for finely defined stringency conditions when using such probes to detect a microorganism of interest.

In an effort to identify target sequences with greater variability, we have investigated the 16s/23s ribosomal spacer sequences of eubacteria. We argued that the spacer (intergenic) regions should be under minimal selective pressure during evolution and therefore should vary more extensively than sequences within genes that have functional roles. By analogy with the general methodology employed for the analysis of the intragenic 16s rRNA regions,[2,3] a general approach is presented that allows one to establish rapidly the DNA sequence of 16s/23s spacer regions of eubacteria and thereby make a logical choice for the best probe of a given organism.

STRATEGY

Ribosomes are essential constituents for eukaryotic and prokaryotic cell proliferation and are composed of protein and RNA moities. In eubacteria, the RNA entity comprises of three distinct types: the 16s, 23s, and 5s rRNAs. The genes coding for these rRNAs exhibit an operon organization that is essentially consistent from one eubacterium to another. Briefly, the operon organization consists of a promoter region followed by a se-

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quence coding for the 16s rRNA, a spacer or intergenic sequence (which in some instances may contain coding sequences for tRNAs), the 23s rRNA coding sequence, another short spacer sequence, and the sequence coding for 5s rRNA. This arrangement is present in the genome in multicopies, and the number of operons present can vary from one group of microorganisms to another. To date, a number of eubacterial ribosomal operons have been partially or completely sequenced, in addition to over 144 16s rRNAs and 13 23s rRNAs.[4,5] Upon examination of the spacer sequences available, we observed that extensive sequence variation existed between microorganisms. It follows that the spacer region should be a good source of species-specific sequences. On the basis of this premise and the sequence data available for 16s and 23s rRNAs that flank spacer regions, we synthesized oligonucleotide PCR primers that could potentially be used in a general fashion to amplify 16s/23s spacer sequences from a wide range of eubacteria. The 5′ primer, A1, corresponds to a conserved sequence motif from the 3′ end of 16s rRNAs (from position 1493 to 1513 of the 16s rRNA of *E. coli*) and the 3′ primer, B1, was deduced from an alignment of the 13 23s 5′ sequences (from position 23 to 43 of the 23s rRNA of *E. coli*) (see Fig. 4). These primers are then used to amplify the spacer regions of the eubacteria of interest; the amplified products are then sequenced and a specific DNA probe or PCR primers can be selected for a particular eubacterium. Figure 1 demonstrates the overall strategy involved.

In this study we have investigated the 16s/23s spacer sequences of a number of species of the genus *Clostridium* to examine the efficacy of the method. The genus *Clostridium* encompasses gram-positive anaerobic endospore-forming eubacteria of broad interest to biotechnology, agriculture, and medicine. To illustrate the approach, we have developed a specific DNA oligonucleotide probe and specific oligonucleotide primers for use in a PCR assay to detect and distinguish *Clostridium perfringens*, a human pathogen, from other clostridia in the genus.

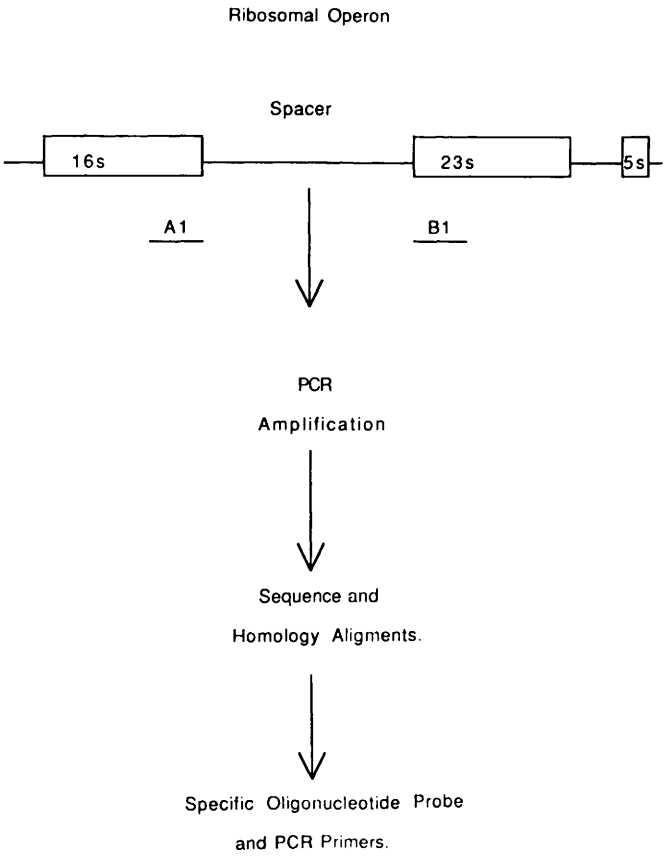

FIGURE 1 Schematic representation of the regime used for developing 16s/23s spacer region-specific oligonucleotide DNA probes and PCR primers for eubacteria.

EXPERIMENTAL PROTOCOL

Enzymes and Reagents

Restriction endonuclease *Sma*I, T4 ligase, T4 polynucleotide kinase, and *Taq* DNA polymerase were purchased from Promega. M13mp11 DNA, proteinase K, RNase, dNTPs, and pBR *Hae*III size markers were purchased from Boehringer-Mannheim. T7 polymerase and sequencing reaction mixes were purchased from Pharmacia. Nytran was purchased from Schleicher & Schuell. NuSieve agarose gel was purchased from FMC Bioproducts. [γ-^{32}P]dATP was purchased from Amersham International.

Oligonucleotides

Single-strand oligonucleotides (all 20-mers) were synthesized on an Applied Biosystems automated synthesizer. After deblocking and ethanol precipitation/washing, no further purification of the oligonucleotides was required prior to use as an oligonucleotide probe or as PCR primers.

Preparation of Total Genomic DNA from *Clostridium* spp. and *B. subtilus*

Total genomic DNA was prepared as follows: 250 ml of clostridial cultures were grown anaerobically at 37°C without agitation in reinforced clostridial medium overnight. The cultures were then harvested by centrifugation at 7000 rpm in a Sorvall GSA rotor at 4°C for 10 min. The supernatants were removed and the pellets were resuspended in 2 ml of sterile deionized water. Then, 8 ml of ice-cold absolute acetone was added to the cellular emulsion and mixed vigorously, and the cells were kept on ice for 30 min. The cells were once again harvested as above and the resultant pellet was allowed to dry under vacuum for 20 min. The cell pellets were then resuspended in 5 ml of 0.1 M Tris-HCl (pH 8.0), lysozyme (10 mg/ml, final concentration), incubated at 37°C for 4 hr under continuous agitation, and then harvested as above. The pellet was

resuspended in 5 ml of sterile water containing 2 mg/ml proteinase K and incubated at 4°C overnight, followed by an incubation at 37°C for 1 hr. One milliliter of 20% SDS and 20 µl of diethylpyrocarbonate (a nuclease inhibitor) were then added and the suspension was allowed to stand at 37°C for a further 15 min. Six milliliters of Tris-HCl–saturated phenol (pH 8.0) was added to the suspension and then centrifuged, and the aqueous phase was removed and extracted again with phenol. A subsequent extraction of the aqueous phase with 1/2 volume phenol, 1/2 volume chloroform/isoamyl alcohol (24:1) was then carried out, the aqueous phase was removed, and six volumes of absolute ethanol added. Total genomic DNA was spooled out with a sterile glass rod, allowed to air-dry, and then resuspended in 1 ml of sterile deionized water containing RNase (10 µg/ml).

PCR Amplification of the 16s/23s Spacer Sequences

PCR amplifications were routinely carried out in a 100-µl reaction volume which consisted of: 100 ng of clostridial total genomic DNA equivalent to 10^5 bacterial cells, 100 ng of each of the primers A1/B1 (Fig. 3), 20 mM NaCl, 50 mM Tris (pH 9), 1% Triton X-100, 0.1% gelatin, 4 mM $MgCl_2$, 200 µM of each of the four dNTPs, and 2.5 units of *Taq* DNA polymerase. Where PCR amplification was carried out directly from culture, the bacterial cells (10^6 or less) were initially heated to 95°C for 5 min, followed by an incubation at 55°C with proteinase K (10 µg/ml) for 5 min and ending with an incubation at 95°C for 10 min to denature the proteinase K.[2] At this point *Taq* DNA polymerase was then added. PCR amplifications were carried out for 30 sec denaturation at 94°C, 30 sec annealing at temperatures ranging from 40°C to 55°C (the annealing temperature of the primers varied for each eubacteria such that background of nonspecific amplified products was eliminated; these values are given in parentheses in Figs. 2 and 3) and an extension time of 30 sec at 72°C. Thirty cycles were carried out in a Perkin-Elmer Cetus Thermocycler. The degree and the specificity of amplification were analyzed by gel electrophoresis on 3% NuSieve agarose minigels. Approximately 50 µl of each PCR reaction was then used for the purposes of cloning into the *Sma*I site of M13mp11.[2] Single-stranded DNA was prepared from recombinants for dideoxy sequencing using T7 polymerase.

PCR Amplification Using the C. *perfringens*-Specific Primers

PCR reactions, using specific primers C1/C2 (these sequences are underlined in Fig. 3), were carried out as above, except that the primers had an optimum $MgCl_2$ final concentration of 3 mM and an optimal annealing temperature of 45°C.

Radioactive Probe Preparation and Hybridization Conditions

One hundred nanograms of the 20-mer DNA probe were end-labeled with [γ-^{32}P]ATP by the recommended procedures using T4 polynucleotide kinase. PCR products were transferred to Nytran using the protocol outlined by the manufacturers. Prehybridization was carried out in 100-ml solution containing 6x SSC, 10x Denhardts, and 0.1% SDS for 1 hr at 65°C. The prehybridization solution was removed and a hybridization solution (8 ml) consisting of 5x SSPE and 0.1% SDS was added. Hybridization was carried out for 1 hr at 50°C. The membranes were then washed two times in 100 ml of 6x SSC, 0.1% SDS for 10 min at room temperature, followed by a final stringent wash of a 100-ml solution consisting of 2x SSC, 0.1% SDS at hybridization temperature (50°C) for 30 min. The membranes were then exposed to X-ray film and autoradiography was carried out for up to 5 hr at −80°C.

RESULTS

The Generation of a Specific DNA Probe and PCR Primers for *Clostridium perfringens*

Initially, primers A1 and B1 were used successfully to amplify a broad range of both gram-positive and gram-negative eubacteria 16s/23s spacer regions using the PCR. The results obtained from the gel electrophoresis pattern of the amplified products show the extent of sequence variation that exists between eubacterial spacer regions, as fragment mobility for each amplified product varied greatly, with a size range of 250 to 600 bp (Table 1; Fig. 2). In an effort to determine whether such sequence variation was observed between closely related eubacteria, PCR amplification of the

TABLE 1 Bacterial Strains Used in This Study

1.	*Micrococcus luteus*	ATCC.9278
2.	*Corynebacterium diphtheriae*	ATCC.27010
3.	*Serratia marcescens*	ATCC.13880
4.	*Mycobacterium tuberculosis*	ATCC. 9360
5.	*Enterococcus faecalis*	ATCC.19433
6.	*Pseudomonas aeruginosa*	ATCC.19582
7.	*Bacillus subtilis*	ATCC. 6051
8.	*Clostridium beijerinckii*	NCDO. 1759
9.	*Clostridium buryricum*	DSM. 552
10.	*Clostridium difficile*	ATCC. 9689
11.	*Clostridium glycolicum*	NCDO. 1791
12.	*Clostridium pasteurianum*	DSM. 525
13.	*Clostridium perfringens*	ATCC.13124
14.	*Clostridium sporogenes*	NCIB. 532
15.	*Clostridium tyrobutyricum*	DSM. 663
16.	*Clostridium barkeri*	ATCC.25843
17.	*Clostridium nexile*	DSM. 1787

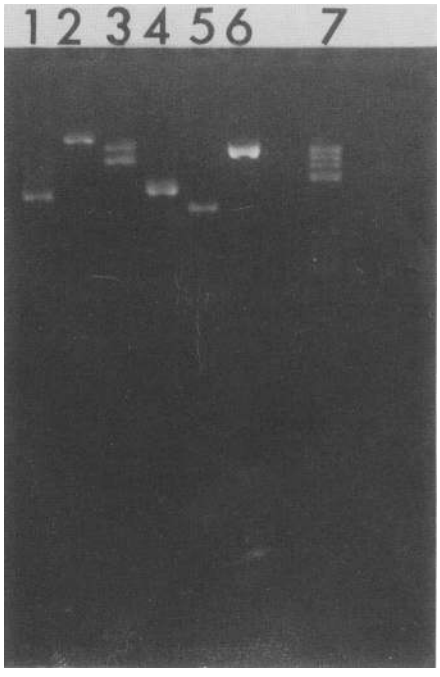

FIGURE 2 Gel electrophoresis of PCR-amplified 16s/23s intergenic spacer regions from a range of eubacteria using general primers A1/B1. (Lane *1*) *M. luteus* (50°C); (lane *2*) *C. diphtheriae* (45°C); (lane *3*) *S. marcesens* (55°C); (lane *4*) *M. tuberculosis* (45°C); (lane *5*) *E. faecalis* (50°C); (lane *6*) *P. aeroguinosa* (55°C); (lane *7*) pBR322 *Hae*III size markers. Optimum annealing temperatures of primers are given in parentheses.

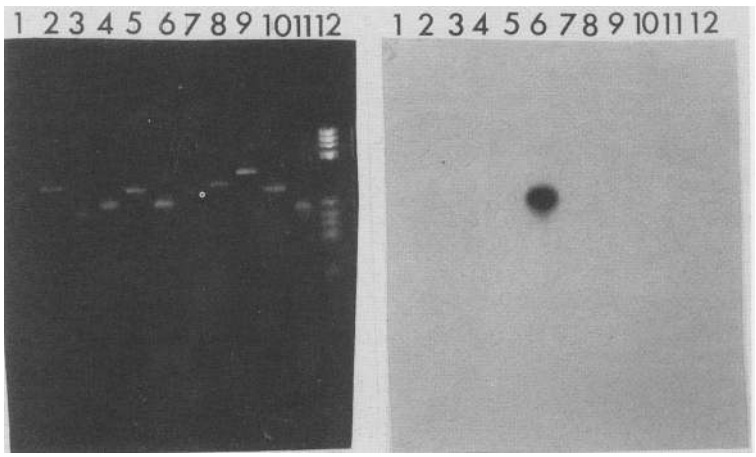

FIGURE 3 (*Left*) Gel electrophoresis (3% NuSieve) of amplified products generated from A1/B1-primed PCR reactions of *C. beijerinckii* (45°C) (lane *1*); *C. butyricum* (45°C) (lane *2*); *C. difficile* (40°C) (lane *3*); *C. glycolicum* (40°C) (lane *4*); *C. pasteurianum* (50°C) (lane *5*); *C. perfringens* (45°C) (lane *6*); *C. sporogenes* (40°C) (lane *7*); *C. tyrobutyricum* (40°C) (lane *8*); *C. barkeri* (55°C)(lane *9*); *C. nexile* (45°C) (lane *10*); *B. subtilis* (45°C) (lane *11*); pBR *Hae*III size markers (lane *12*). Optimum annealing temperatures for each microorganism with primers A1/B1 are given in parentheses. (*Right*) Corresponding Southern blot demonstrating the specificity of the *C. perfringens* oligonucleotide probe (see Fig. 4).

spacer region of 10 clostridia (Table 1) and the phylogenetically related *Bacillus subtilis* was performed using these primers. Again, significant size differences are found (Fig. 3), implying sequence variation. The DNA sequences amplified from three clostridial species were determined. Alignment of these sequences required the introduction of multiple gaps to maximize the homology, which in any case was low, and many opportunities are thus presented in the choice of species-specific probes (Fig. 4).[6] A DNA probe was selected for *C. perfringens* (underlined in Fig. 4) on the basis of these data and this was tested against the original panel of amplified clostridia 16s/23s spacer sequences. Only *C. perfringens* was detected with this oligonucleotide probe (Fig. 3b) under moderate stringency conditions. The DNA sequence in Figure 4 also allows one to select species-specific primers for selective PCR amplification. When these were chosen for *C. perfringens* and tested against the panel of clostridia, only *C. perfringens* was amplified, as judged by visualization with ethidium bromide staining of DNA following gel electrophoresis (Fig. 5). In a final demonstration of the application of this technique, six clinical isolates that had been identified as *C. perfringens* using standard microbiological tests were amplified with the species-specific primers. One sample failed to yield a visible band (Fig. 6A). When the general primers (A1 and B1) were used, a DNA fragment of a size greater than the control and the other *C. perfringens* amplified fragments was obtained for this sample (Fig. 6B). The results suggested that this isolate was not *C. perfringens*. A final indication that this isolate was not *C. perfringens* comes from the fact that all the other samples hybridized with the *C. perfringens* oligonucleotide probe following amplification (Fig. 6C), whereas it did not.

Analysis of Multiple Copies of the C. *perfringens* 16s/23s Spacer Sequence

It is known that *C. perfringens* contains nine copies of the ribosomal operon.[7] In an effort to address the question of whether sequence variation between the 16s/23s spacer regions of each of these operons existed and if tRNA coding sequences were present, we sequenced 12 independent *C. perfringens* M13 clones derived from fragments amplified by PCR using the general primers.

Six of these recombinants were shown to have identical sequences, four were shown to exhibit one or more point substitutions, concentrated predominantly at the 3′ end of the PCR products, and two were shown to contain deletions, 20 and 56 bases, respectively, also at the 3′ end of the PCR products. Each substitution or set of substitutions was unique to a single PCR product, indicating that such sequence alterations were random events and are presumably due to "errors" of

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A1:  5'AGTCGTAACAAGGTAAGCCG3'
B1:  5'C T/C A/G T/C TGCCAAGCATCCACT3'

                                                                  C1.
C.perfringens   AGGAGAACCTGCGGCTGGATCACCTCCTTTCTAAGGAATACATCT--TAGGACAACTAAG
C.pasteurianum  AGGAGAACCTGCGGCTGGATCACCTCCTTTCTAAGGAGTAATTGTAGCAGGATAACTGTT
C.difficile     AG-AGAACCTGCCGGTGAATCACCTCCTTTCTAAGGAGAA----TAGAAAGAAGAAAATT
                ** ********* * ** ********************  *    *   * **  *

C.perfringens   ATG-ATAATGAATTCTGGATAATATCTCTGTTTAATTTTGAGAGACTATCTCTCA-AAAT
C.pasteurianum  GTATACATTGGTTTCTTACTCTTGTCTCTGTTTAATTTTGAGAGATCAGTTCTCTTAAAA
C.difficile     CTTTCTAAAGG---CTGAAT----TCTCTGTTTAATTTTGAGAGACCATTCTCTCAAAAT
                 *    *  *    **   *    **********************  *        ***

C.perfringens   TGTT-CTTTGAA-AATTGCACA-----------TAATTTAA--------TTTATAGA-A-
C.pasteurianum  TGTA-CTTTGAA-AATTGCATAGAGAAACAAAGTAAAGTAAAATAATCCTTTGTATA-AT
C.difficile     TGAAACTTCTAATAAAATTGGGAAGTAGCTGATCATCACCAAATCGTAAATTTTGGATGC
                **   ***  ** **                    *     *         ** *  *

C.perfringens   ACAAC---AAGCC--AAA--TTGG----CAAAACCAATTT---CTATTCTTTGTAAAATG
C.pasteurianum  ATGATTTTAATCG--AAAGATTGAA-ATTAAAACAAATAAAGACTAAGCTCTAAAACGTA
C.difficile     CTAGCTACGTTCTTTGAAAATTGCACAGTGAATAAAGTAAAGCTAAAGGTATATAAAA-A
                        *    **  ***       **   * *       *   * *  **
                     C2.
C.perfringens   AGAACTATAAC--T-AATATA-GGTCAAGCTACAAAGGGCGCAT
C.pasteurianum  ACGCCTAAAAGAGT-AACA-A-GGTCAAGCTACAAAGGGCGCAT
C.difficile     TCCTTTGTAAGAATCAATTTAAGGTCAAGCTACAAAGGGCGCAT
                     *  **   * **   * **********************
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FIGURE 4 Multiple sequence alignment of the 16s/23s spacer region of *C. perfringens*, *C. pasteurianum*, and *C. difficile*. The specific *C. perfringens* oligonucleotide probe is underlined, as are the specific primers C1/C2. The sequences of *C. pasteurianum* and *C. difficile* were obtained from 10 different M13 isolates. Where random base point alterations were noted the most frequently occurring base is reported.

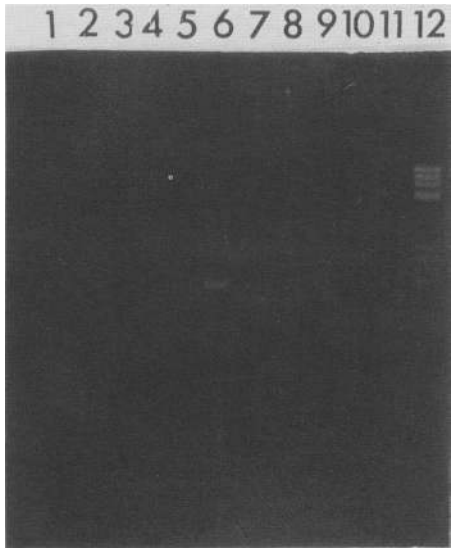

FIGURE 5 Specificity of PCR amplification with *C. perfringens* spacer primers C1/C2. These primers were assayed against the panel of clostridia used in this study. (Lane *1*) *C. heijerinckii*; (lane *2*) *C. butyricum*; (lane *3*) *C. difficle*; (lane *4*) *C. glycolicum*; (lane *5*) *C. pasteurianum*; (lane *6*) *C. perfringens*; (lane *7*) *C. sporogenes*; (lane *8*) *C. tyrobutyricum*; (lane *9*) *C. barkeri*; (lane *10*) *C. nexile*; (lane *11*) *B. subtilis*; (lane *12*) pBR322 *Hae*III size markers.

PCR amplification. Recently, similar observations have been published in more detail[8] and our results are consistent with the error type and error rate of PCR amplification obtained. It is suggested that there is a requirement to sequence at least eight to ten independent PCR products, with three to four of these products having identical sequences, such that an overall correct consensus sequence can be determined.[8]

From this analysis we have concluded that the 16s/23s spacer region of each of the nine operons of *C. perfringens* is very similar in sequence. Furthermore, no tRNA sequences were evident with these sequences. This suggests that within a microorganism there is strong pressure for this potentially hypervariable region to remain constant.

Preliminary evidence to suggest that tRNA sequences may be present in *S. marcesens* 16s/23s spacer sequences is given in Figure 2 (lane 3), as two PCR products are observed upon amplification with the general primers A1/B1. These amplified products differ by approximately 80 bp in length, which would correspond to the presence of at least one tRNA sequence in the intergenic regions of some of the 16s/23s operons of this microorganism.

DISCUSSION

In an earlier study, we outlined a general method that can be used to develop DNA probes for microorganisms.[2] In its original presentation, the strategy was shown to work effectively, focusing on the variable V6 region of 16s rRNA. However within closely related organisms (e.g., Mycobacteria species) this "variable" region is quite invariant and therefore is not a suitable target for the generation of DNA probes.[9] We have, as a result, sought to identify more hypervariable regions using the strategy originally developed for the 16s rRNA variable intragenic regions. Using this methodology, we have demonstrated that very significant sequence heterogeneity exists within the eubacterial 16s/23s spacer region at the genus and species levels. In a similar approach recently reported, the corresponding spacer region of closely related symbiotic fungi suggests that the degree of heterogeneity in these genera is not very marked.[10] However, further studies that would provide data on this region of a greater sample of fungi would be required to clarify this matter.

Given the high degree of sequence variation observed from one species to another, one concern was that the spacer region might alter significantly between different strains of a species. In a limited experiment, we tested our specific oligonucleotide probe and PCR primers against a panel of six different clinical isolates of *C. perfringens*. This microorganism causes a number of pathological conditions of man and animals that can be broadly defined as necrotic diseases (i.e., gas gangrene) and enterotoxemias (i.e., food-poisoning syndromes). The clinical samples that were used to validate this probe were all presumptive *C. perfringens* on the basis of their properties in standard clinical microbiological tests. Five of the isolates were confirmed as being *C. perfringens* on the basis of the successful use of the specific probe and primers that had been developed in the course of this study from data obtained from the *C. perfringens* ATCC 13124 typed strain. This gives some indication that these sequences are conserved from one strain to another; however, a more extensive study is required to validate such observations. The clinical isolate, which was not detected by the specific DNA probe/specific PCR primers, was reevaluated by carrying out three phenotypic tests to determine whether this isolate was in fact a *C. perfringens*. Upon reassessment, this microorganism was unable to hydrolyze 10% gelatin, did not liquify esculin, and was a faculative anaerobe.[11] Consequently this culture, in fact, was not

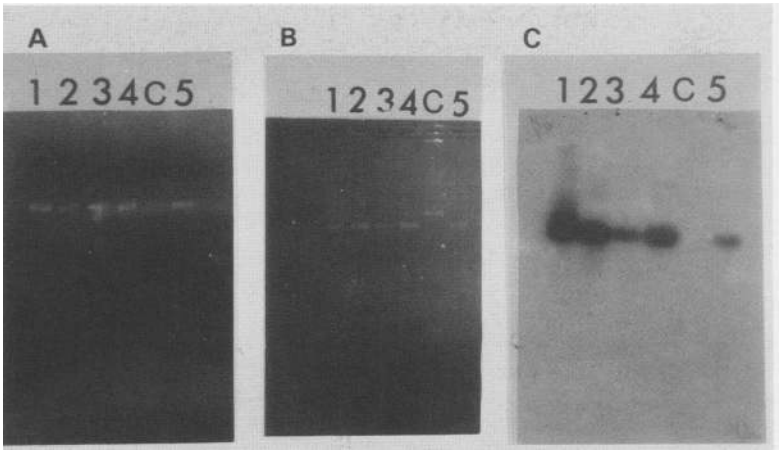

FIGURE 6 (*A*) PCR amplification of six clinical isolates, identified as *C. perfringens*, using specific primers C1/C2. (*B*) Subsequent PCR amplification of the six clinical isolates using general primers A1/B1. (*C*) Corresponding Southern blot using the *C. perfringens*-specific probe.

C. perfringens and was considered to have arisen from process contamination.

The overall strategy used in this and the previous study[2] has the advantage of providing specific DNA probes for each microorganism from the panel of organisms tested. For example the data shown in Figure 4 of this study allow for the generation of DNA probes/PCR primers for *C. pasteurianum* and for *D. difficle*. The latter, like *C. perfringens*, is a human pathogen.[12] This list of probes can be rapidly expanded to other microorganisms using the same rationale. Furthermore, the rapid technique presented requires no previous information of the molecular biology of the microorganism of interest. In conclusion, the use of DNA sequences in the spacer region between the 16s and 23s RNA shows that an efficient and specific DNA probe can be generated that can distinguish readily between closely related eubacteria.

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ERRATUM

Barany, F. 1991. The ligase chain reaction in a PCR world. *PCR Methods Applic.* **1**:5–16.

Table 1 of the above titled paper inadvertently listed an incorrect value for NAD in Method 1; the correct value is 1 mM, not 10 mM as stated. The correct version of Method 1 is reproduced on this page.

TABLE 1 Ligase Chain Reaction Methods

	Method 1[a]
Target DNA	β^A,β^S
Standard detection	1–10 attomoles (6×10^5 to 6×10^6 molecules)
Signal-to-noise no target under standard conditions	1700 to >2000[b]
single-base mismatch under standard conditions	75 to >500[b]
Lowest detection	200 molecules
Position of discriminating nucleotide	3′ base of both strands (single-base 3′ overhang)
T_m discrimination oligonucleotides	64°C–68°C (23- to 28-mers)
T_m adjacent oligonucleotides	70°C (22-mers)
Amount of each oligonucleotide	40 femtomoles (0.28 ng)
Volume	10 μl
Buffer conditions	20 mM Tris-HCl, pH 7.6[c] 100 mM or 150 mM KCl 10 mM $MgCl_2$ 10 mM DTT 1 mM NAD^+ 1 mM EDTA
Carrier DNA to suppress background	4 μg of salmon sperm DNA
Additional features for suppression of target independent background	5′ phosphate on adjacent oligonucleotides only; noncomplementary tails on outside of oligonucleotides; single-base 3′ overhang on discriminating oligonucleotides
Thermostable enzyme	15 nick-closing units[d]
Cycle conditions	94°C, 1 min 65°C, 4 min 20 or 30 cycles or 94°C, 0.5 min 65°C, 2 min 30 or 40 cycles

ERRATUM

Barry, T., G. Colleran, M. Glennon, L.K. Dunican, and F. Gannon. 1991. The 16s/23s ribosomal spacer region as a target for DNA probes to identify eubacteria. *PCR Methods Applic.* **1**:51–62.

Figure 4 has errors in the A1 and B1 primers. The correct primers are:
A1 5′-AGTCGTAACAAGGTAGCCG-3′
B1 5′-C T/C A/G T/C TGCCAAGGCAT CCACC-3′

The 16s/23s ribosomal spacer region as a target for DNA probes to identify eubacteria.

T Barry, G Colleran, M Glennon, et al.

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