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Discrimination of Epidemic and Sporadic Isolates of *Arcobacter butzleri* by Polymerase Chain Reaction-Mediated DNA Fingerprinting

P. VANDAMME,[1,2]* B. A. J. GIESENDORF,[3,4] A. VAN BELKUM,[3] D. PIERARD,[5] S. LAUWERS,[5] K. KERSTERS,[2] J.-P. BUTZLER,[6] H. GOOSSENS,[1] AND W. G. V. QUINT[1,3]

Department of Microbiology, University Hospital, Antwerp,[1] Department of Microbiology, University of Ghent, Ghent,[2] and Department of Microbiology, Academisch Ziekenhuis, Vrije Universiteit Brussel,[5] and World Health Collaborating Center for Enteric Campylobacter, St. Pieter University Hospital,[6] Brussels, Belgium, and Diagnostic Center SSDZ, Delft,[3] and State Institute for Quality Control of Agricultural Products, Wageningen,[4] The Netherlands

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DNA polymorphisms of *Arcobacter butzleri* outbreak-related strains and *Arcobacter* reference strains were determined by use of the polymerase chain reaction with primers aimed at repetitive sequences. The epidemiological relationship among 14 outbreak-related strains was substantiated, as they showed virtually no genomic variations. Their DNA amplification patterns were, however, clearly different from those of all *Arcobacter* reference strains studied; each reference strain was characterized by a unique DNA fingerprint.

We recently described an outbreak of recurrent abdominal cramps which occurred in a nursery and primary school in the Rovigo area in Italy (10). All outbreak-related strains were compared by whole-cell protein analysis, conventional phenotypic tests, and serotyping. In each of these approaches, all outbreak-related strains were identical, suggesting an epidemiological relationship. A representative strain from the group was identified as *Arcobacter butzleri* by use of various genotypic and phenotypic taxonomic methods (10, 11). The genus *Arcobacter* was created recently to include a number of aerotolerant campylobacters (11). These organisms are primarily differentiated from campylobacters by their ability to grow in air and at 15°C (11). Two *Arcobacter* species are found in association with human disease, although their pathological role is poorly understood (10). Most of the human isolates are *A. butzleri*, which is mainly isolated from stool specimens of patients with diarrhea (10).

In the present study, we evaluated the usefulness of polymerase chain reaction (PCR)-mediated detection of DNA polymorphisms with primers aimed at variable sequence motifs (1, 5, 12). This PCR-based typing technique is fast and reliable and appears increasingly useful for the differentiation of strains within species (1, 5, 7–9). As such, the method is extremely useful for the genetic typing of epidemiologically related strains. Fourteen outbreak-related *A. butzleri* strains were compared with 10 additional *A. butzleri* strains isolated from various geographical locations: Canada (LMG 10220, LMG 10223, LMG 10240, LMG 10243, and LMG 11632), Belgium (LMG 10902 and LMG 12107), Northern Ireland (LMG 9906), the United Kingdom (LMG 6620), and the United States (LMG 10828^T). These reference strains were isolated from different sources, including human blood (LMG 6620 and LMG 11632) and feces (LMG 10828^T and LMG 12107), equine (LMG 10240), porcine (LMG 10220), and bovine (LMG 10243) feces, bovine (LMG 10223) and porcine (LMG 9906) fetuses, and an environmental source (LMG 10902). Representative strains of the other *Arcobacter* species, i.e., *A. skirrowii* (LMG 6621^T), *A. cryaerophilus* (LMG 9904^T and LMG 10829, one strain of each of two subgroups [11]), and *A. nitrofigilis* (LMG 7604^T), were also included as reference strains. All strains have been deposited in the University of Ghent Laboratory for Microbiology (LMG) culture collection (11). PCR fingerprinting required 10 to 100 ng of genomic DNA per assay. The DNA amplification patterns were highly reproducible, as variations in the DNA isolation procedures (2, 6, 11) did not affect the DNA profiles (data not shown). The PCR was performed as described before (3). In brief, the reaction mixtures consisted of 10 mM Tris-HCl (pH 9), 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% gelatin, and 0.1% Triton X-100. Deoxyribonucleotide triphosphates were each used at a final concentration of 0.2 mM. Per reaction mixture, 0.625 U of *Taq* DNA polymerase (Sphaero-Q, Leiden, The Netherlands) was added. Four different primers or primer combinations were evaluated with respect to their applicability for strain differentiation: the enterobacterial repetitive intergenic consensus (ERIC) motifs 1R and 2 (5′-ATGTAAGCTCCTGGG GATTCAC-3′ and 5′-AAGTAAGTGACTGGGGTGAGCG-3′, respectively) (12), the repetitive extragenic palindromic (REP) elements 1R and 2 (5′-IIIICGICGICATCIGGC-3′ [I represents inosine, which contains hypoxanthine, a purine base capable of forming Watson-Crick base pairs with A, G, C, or T] and 5′-ICGICTTATCIGGCCTAC-3′, respectively) (12), and the arbitrary primers 1266 (5′-NNNAACAGCTAT GACCATG-3′ [N indicates equimolar mixtures of A, T, G, and C]) and 1267 (5′-GAGCGGCCAAAGGGAGCAGAC-3′) (1). Per reaction mixture, 50 pmol of ERIC or REP primers or 100 pmol of primer 1266 or 1267 was present. PCR conditions for ERIC and REP primers consisted of an initial denaturation step for 5 min, 40 cycles of consecutive denaturation (1 min at 94°C), annealing (1 min at 25°C), and DNA chain extension (4 min at 74°C), and a final elongation step

* Corresponding author.

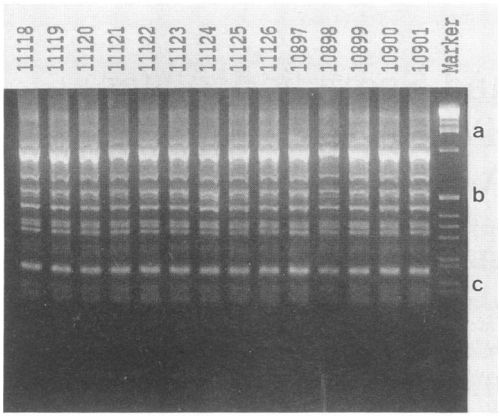

FIG. 1. DNA fingerprinting profiles of *A. butzleri* outbreak-related strains with ERIC 1R and 2 sequences as primers. All strain numbers are LMG numbers. The marker lane displays a 1-kb DNA ladder (GIBCO BRL). The DNA fragments labeled a, b, and c are 1,636, 517 and 506, and 134 bp in length, respectively.

for 10 min at 74°C. PCR conditions for primers 1266 and 1267 consisted of 4 cycles of 5 min at 94°C, 5 min at 40°C, and 5 min at 72°C, 30 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C, and a final elongation step for 10 min at 72°C. The PCR apparatus used was a Biomed model 60 thermocycler. DNA fragments were separated in 2% agarose gels and visualized by ethidium bromide staining. The interpretation of the DNA fingerprints was carried out by visual examination.

When *A. butzleri* DNA was used as a template, few or no DNA fragments were amplified with the REP primers, while a nondiscriminatory profile consisting of two DNA fragments only was obtained with primer 1266 (results not shown). The application of the ERIC primers and primer 1267 yielded complex patterns. The sizes of the DNA fragments generated by the amplification process were between 130 and 2,000 bp (Fig. 1 and 2). The DNA fingerprints of all *Arcobacter* strains had a DNA band of about 130 bp in common when the ERIC 1R and 2 primers were used (Fig. 1 and 2) and a DNA band of about 340 bp when primer 1267 was used (data not shown). These DNA fragments might be used as genus-specific probes, as has been described for the genus *Campylobacter* (3). When the ERIC 1R and 2 primers were used, the DNA patterns generated by the PCR were identical for all outbreak-related strains (Fig. 1). When primer 1267 was used, 13 of 14 strains were again characterized by identical DNA amplification patterns. The DNA banding pattern of strain LMG 11124 contained one additional fragment with an approximate length of 750 bp (results not shown). The overall DNA pattern of the outbreak-related strains was clearly different from the patterns of all other *A. butzleri* strains and reference strains of other *Arcobacter* species (Fig. 2). Each of these reference strains (including *A. butzleri* isolates) was characterized by a unique DNA fingerprint. It is clear that the possibility of recognizing an individual strain offers considerable advantages over classical typing methods, such as biotyping or serotyping, in which unrelated strains regularly belong to the same type. Kiehlbauch et al. (4) reported that ribotyping of arcobacters yielded comparable results. However, this technique involves restriction endonuclease digestion of DNA, electrophoresis and blotting of DNA fragments, hybridization with a probe, and autoradiography or a corresponding revelation method (4).

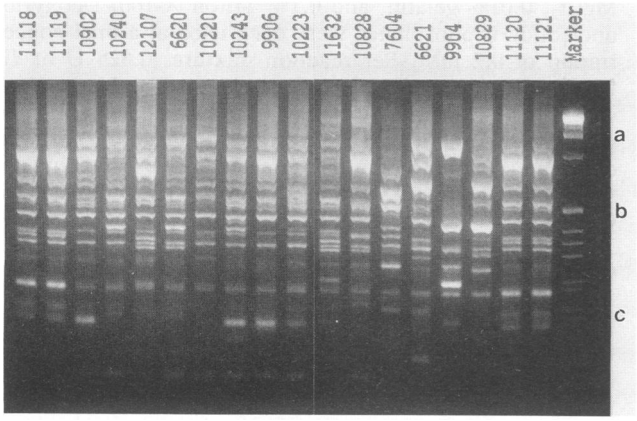

FIG. 2. DNA fingerprinting profiles of *A. butzleri* outbreak-related strains (LMG 11118, LMG 11119, LMG 11120, and LMG 11121) and *Arcobacter* reference strains (*A. nitrofigilis* LMG 7604^T, *A. skirrowii* LMG 6621^T, *A. cryaerophilus* LMG 9904^T and LMG 10829, and *A. butzleri* reference strains) with ERIC 1R and 2 sequences as primers. Strain numbers, molecular length markers, and DNA fragments are as given for Fig. 1.

All 14 isolates obtained from the feces of children with abdominal cramps were considered to belong to a single outbreak: all patients presented with similar symptoms, and all isolates were identical in the biochemical, serological, and protein electrophoretic analyses (10). This epidemiological relationship was further substantiated by the results of the present study. Obviously, all 14 isolates are derived from a single clone that caused the outbreak.

PCR-mediated DNA fingerprinting offers advantages over classical and other DNA-based typing techniques. A minimal amount of DNA is required. An organism from any taxon, prokaryotic or eukaryotic, can be typed with the same equipment and reagents, provided that suitable primers are selected in a preliminary analysis. The meaning of additional DNA bands, such as the one that we found for strain LMG 11124 with primer 1267, is unclear. An evaluation of this PCR-based typing method versus well-known typing techniques will help to solve such problems.

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REFERENCES

1. **Akopyanz, N., N. O. Bukanov, T. U. Westblom, S. Kresovich, and D. E. Berg.** 1992. DNA diversity among clinical isolates of *Helicobacter pylori* detected by PCR-based RAPD fingerprinting. Nucleic Acids Res. **20**:5137–5142.
2. **Giesendorf, B. A. J., W. G. V. Quint, M. H. C. Henkens, H. Stegeman, F. A. Huf, and H. G. M. Niesters.** 1992. Rapid and sensitive detection of *Campylobacter* spp. in chicken products by using the polymerase chain reaction. Appl. Environ. Microbiol. **58**:3804–3808.
3. **Giesendorf, B. A. J., A. Van Belkum, A. Koeken, H. Stegeman, M. H. C. Henkens, J. Van Der Plas, H. Goossens, H. G. M. Niesters, and W. G. V. Quint.** 1993. Development of species-specific DNA probes for *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter lari* by polymerase chain reaction fingerprinting. J. Clin. Microbiol. **31**:1541–1546.

4. **Kiehlbauch, J. A., B. D. Plikaytis, B. Swaminathan, D. N. Cameron, and I. K. Wachsmuth.** 1991. Restriction fragment length polymorphisms in the ribosomal genes for species identification and subtyping of aerotolerant *Campylobacter* species. J. Clin. Microbiol. **29:**1670–1676.
5. **Lupski, J. R., and G. E. Weinstock.** 1992. Short, interspersed repetitive DNA sequences in prokaryotic genomes. J. Bacteriol. **174:**4525–4529.
6. **Pitcher, D. G., N. A. Saunders, and R. J. Owen.** 1989. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett. Appl. Microbiol. **8:**151–156.
7. **Riley, D. E., M. Samadpour, and J. N. Krieger.** 1991. Detection of variable DNA repeats in diverse eukaryotic microorganisms by a single set of polymerase chain reaction primers. J. Clin. Microbiol. **29:**2746–2751.
8. **Van Belkum, A., R. Bax, P. Peerbooms, W. H. F. Goessens, N. Van Leeuwen, and W. G. V. Quint.** 1993. Comparison of phage typing and DNA fingerprinting by polymerase chain reaction for discrimination of methicillin-resistant *Staphylococcus aureus* strains. J. Clin. Microbiol. **31:**798–803.
9. **Van Belkum, A., J. De Jonckheere, and W. G. V. Quint.** 1992. Genotyping *Naegleria* spp. and *Naegleria fowleri* isolates by interrepeat polymerase chain reaction. J. Clin. Microbiol. **30:** 2595–2598.
10. **Vandamme, P., P. Pugina, G. Benzi, R. Van Etterijck, L. Vlaes, K. Kersters, J.-P. Butzler, H. Lior, and S. Lauwers.** 1992. Outbreak of recurrent abdominal cramps associated with *Arcobacter butzleri* in an Italian school. J. Clin. Microbiol. **30:**2335–2337.
11. **Vandamme, P., M. Vancanneyt, B. Pot, L. Mels, B. Hoste, D. Dewettinck, L. Vlaes, C. Van Den Borre, R. Higgins, J. Hommez, K. Kersters, J.-P. Butzler, and H. Goossens.** 1992. Polyphasic taxonomic study of the emended genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. Int. J. Syst. Bacteriol. **42:**344–356.
12. **Versalovic, J., T. Koeuth, and J. R. Lupski.** 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Res. **19:** 6823–6831.