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Arbitrarily Primed Polymerase Chain Reaction Provides Rapid Differentiation of *Proteus mirabilis* Isolates from a Pediatric Hospital

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During a systematic survey, maternal carriage of *Proteus mirabilis* was found over a 25-day period in 18 pregnant women admitted to the delivery ward of our hospital maternity. Five neonates born to these mothers were found to be colonized with *P. mirabilis*. We report here on the use of DNA fingerprinting by the arbitrarily primed polymerase chain reaction technique (AP-PCR) for the epidemiological investigation of this sudden outbreak. This approach was compared with the analysis of restriction fragment length polymorphisms of ribosomal DNA regions (ribotyping). Results of the AP-PCR and of ribotyping were in complete agreement in showing the genetic unrelatedness of the isolates obtained from each mother. Moreover, the results showed mother-to-infant vertical transmission of *P. mirabilis* in the neonates. AP-PCR is a rapid and discriminative method which seems particularly well suited to the epidemiological study of *P. mirabilis*.

Proteus mirabilis is an uncommon etiologic organism for neonatal bacterial meningitis (15). Neonatal meningitis caused by *P. mirabilis* is often accompanied by brain abscesses or ventriculitis and carries high mortality and morbidity rates. Identification of *P. mirabilis* relies upon phenotypic characteristics such as biochemical types, antimicrobial susceptibility profiles (6), serotypes (7), susceptibility to phage lysis (10), production of and sensitivity to proticin, and Dienes types (7, 10). Although these methods have provided useful information (13, 18), they are tedious to perform and not without problems (10).

Nosocomial outbreaks involving *P. mirabilis* have been reported infrequently (5, 6, 21), probably because typing procedures to demonstrate transmission of this organism are either inadequate or unavailable (10). To overcome the uncertainties associated with phenotypic assays, recent epidemiological studies of other species have emphasized the use of DNA-based methodologies.

During a systematic survey for the maternal carriage of *Streptococcus agalactiae*, we found 18 pregnant women to be colonized with *P. mirabilis* over a 25-day period in our maternity at the Hôpital Robert Debré in Paris, France. Five neonates born to these mothers were found to be colonized with *P. mirabilis*. Because of the potential danger of *P. mirabilis* in newborns, an epidemiological investigation was undertaken. We report here on the use of DNA fingerprinting by the recently introduced arbitrarily primed polymerase chain reaction technique (AP-PCR) (23, 25) for the epidemiological investigation of this sudden outbreak. This approach was compared with the analysis of restriction fragment length polymorphisms (RFLPs) of ribosomal DNA (rDNA) regions (ribotyping) and with conventional systems, such as biochemical and antimicrobial susceptibility profiles.

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MATERIALS AND METHODS

Patients. For the systematic surveillance of *S. agalactiae* maternal carriage, vagina samples are collected from every woman admitted to the delivery ward of our hospital's maternity. We take an average of 405 samples per month. Samples were taken from the posterior wall of the vagina with a speculum positioned. Altogether, 23 patients colonized with *P. mirabilis* were studied. Eighteen were pregnant women who were found to be vaginal carriers of *P. mirabilis*. Five were neonates, including a pair of twins, born to four of these women. The neonates were considered colonized on the basis of clinical and biological parameters and received no antibiotic treatment.

Bacterial strains. A total of 29 clinical isolates of *P. mirabilis* and the reference strain ATCC 29906 were selected for the study. The clinical isolates were obtained over a period of 25 days from the vaginas of the 18 mothers and from the ears, gastric aspirates, and umbilici of the five newborns taken at birth (Table 1).

Isolates were identified as *P. mirabilis* by the analytical profile index procedure API 20E (API, La Balme les Grottes, France). Susceptibility of the organisms to antimicrobial agents was studied by the disk diffusion method.

AP-PCR. Total *P. mirabilis* DNA was prepared by a method described previously (4). The single PCR primer, used for the AP-PCR procedure (5'-TCACGATGCA-3'), was one of those proposed by Williams et al. (25). PCR was carried out in 50 μl of a 100 mM Tris-HCl (pH 8.3) buffer containing 50 mM KCl, 4 mM $MgCl_2$, 0.4 mM each deoxynucleoside triphosphate, 3 μM primer, 50 ng of DNA, and 2.5 U of *Taq* DNA polymerase (Beckman, Fullerton, Calif.). AP-PCR was performed in a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.) programmed for 35 cycles, as described previously (25). Amplification products were resolved by electrophoresis on a 2% agarose gel and detected by staining with ethidium bromide (2).

TABLE 1. Phenotypic and genotypic characteristics of the *P. mirabilis* strains studied

Strain[a]	Date of isolation (August)	Origin of strain	API profile	Ampicillin susceptibility[b]	rDNA RFLP pattern		Ribotype	AP-PCR profile
					*Eco*RI	*Hin*dIII		
1M	5	Vagina	0736	S	a	a	A	1
2M	6	Vagina	0736	S	b	b	B	2
3M	12	Vagina	0136	R	c	c	C	3
3NB1-1	12	Ear	0136	R	c	c	C	3
3NB1-2	12	Gastric aspirate	0136	R	c	c	C	3
3NB2-1	12	Ear	0136	R	c	c	C	3
3NB2-2	17	Umbilicus	0136	R	c	c	C	3
4M	12	Vagina	2536	R	d	d	D	4
4NB-1	12	Gastric aspirate	2536	R	d	d	D	4
4NB-2	12	Ear	2536	R	d	d	D	4
4NB-3	17	Right eye	2536	R	d	d	D	4
4NB-4	17	Left eye	2536	R	d	d	D	4
4NB-5	17	Umbilicus	2536	R	d	d	D	4
5M	13	Vagina	0536	R	e	e	E	5
6M	13	Vagina	0536	R	f	f	F	6
6NB	13	Gastric aspirate	0536	R	f	f	F	6
7M	17	Vagina	0136	S	d	g	G	7
8M	18	Vagina	0536	S	g	h	H	8
9M	18	Vagina	0736	S	h	i	I	9
10M	19	Vagina	0736	R	i	j	J	10
11M	23	Vagina	0536	S	j	k	K	11
12M	25	Vagina	0536	S	c	l	L	12
13M	25	Vagina	0536	S	k	m	M	13
14M	23	Vagina	0736	S	l	n	N	14
15M	23	Vagina	0136	R	m	o	O	15
15NB	23	Gastric aspirate	0136	R	m	o	O	15
16M	29	Vagina	0536	S	n	p	P	16
17M	28	Vagina	0536	S	o	q	Q	17
18M	30	Vagina	0736	S	p	r	R	18

[a] Strain numbering is as follows: the first number refers to a given mother or mother-newborn pair; M and NB refer to strains isolated from mothers and newborns, respectively; NB1 and NB2 are twins; the final number corresponds to different isolates from the same individual.
[b] S, susceptible; R, resistant.

RFLP analysis. DNA (4 μg) was digested with *Hin*dIII and *Eco*RI restriction enzymes (Boehringer, Mannheim, Germany) according to the manufacturer's specifications and analyzed by electrophoresis on 0.8% ethidium bromide-containing submarine agarose gels in a 0.04 M Tris-acetate–0.001 M EDTA buffer. The DNA fragment size marker Raoul I (Appligene, Strasbourg, France) was used. Size-separated restriction fragments were then transferred to a nylon membrane (GeneScreen Plus; New England Nuclear Products, Boston, Mass.) by the method of Southern. 16 and 23S rRNA from *Escherichia coli* (Boehringer) was used as a probe and cold labelled by random oligopriming with a mixture of hexanucleotides (Pharmacia, Uppsala, Sweden) and cloned mouse mammary leukemia virus reverse transcriptase (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) in the presence of 0.35 mM digoxigenin-11-dUTP (Boehringer). DNA hybridization temperature and chemiluminescence detection procedures were as described previously (3).

RESULTS

For 25 days, during August, *P. mirabilis* was found in vagina samples from 18 pregnant women. The incidence in the maternity during the epidemic period was 4.6% (18 of 390 patients). This was significantly higher than the mean rate of 1.9% during the 6 preceding months ($P < 0.01$). Such a high rate was not noted for the previous years, regardless of the season. Of the 19 neonates (including a pair of twins) born to these mothers, 5 were found to be colonized by *P. mirabilis*.

Four biochemical patterns were observed among the 29 clinical isolates studied. Twelve isolates were sensitive to ampicillin, and 17 isolates were resistant to this antibiotic. The combination of these two phenotypic traits resulted in seven different phenotypic patterns (Table 1).

In contrast, within the 29 clinical isolates, AP-PCR generated 18 different patterns. A specific pattern was obtained for each strain from each woman (Fig. 1A), but identical patterns were found for all the strains isolated from a given mother-newborn pair (Fig. 1B). The reference strain produced still another pattern. Eighteen different ribotypes were defined on the basis of the 18 rDNA RFLP patterns generated by *Hin*dIII (Fig. 2). *Eco*RI was less discriminant, producing only 16 different patterns. Thus, distinct ribotypes were obtained for each strain from each woman. Identical ribotypes were found for the strains obtained from each newborn and its mother, demonstrating mother-to-infant vertical transmission for the twins and the three other neonates (Table 1).

Results of the two genotypic approaches were therefore in complete agreement in showing the genetic unrelatedness of all the strains obtained from the pregnant women and the vertical transmission of *P. mirabilis* from the mother of each colonized infant.

DISCUSSION

P. mirabilis is a rare but not exceptional organism of the vaginal flora. In our maternity, we usually found it in 1.9% of the vaginal cultures. It has been reported to be responsible

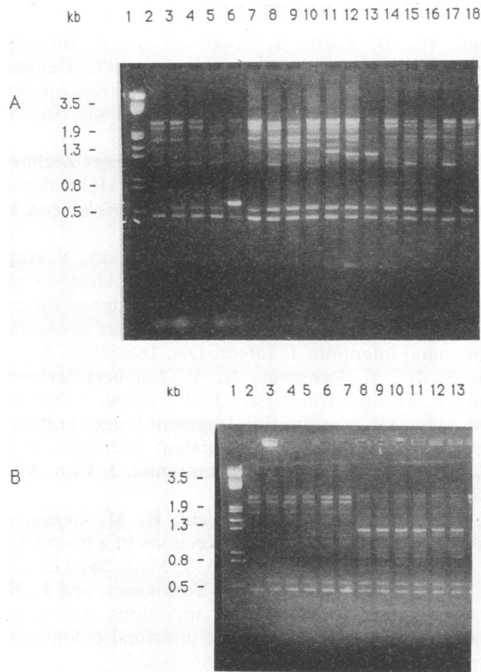

FIG. 1. *P. mirabilis* DNA fingerprinting by AP-PCR. (A) Isolates from 16 of the 18 pregnant women (lanes 3 to 18). Lane 1, size marker; lane 2, strain ATCC 29906. All the patterns are different. (B) Mother-to-infant transmission in two of the pregnant women. Lane 1, size marker; lane 2, strain ATCC 29906; lane 3, vaginal sample from patient 3 (strain 3M); lanes 4 and 5, ear sample (strain 3NB1-1) and gastric aspirate (strain 3NB1-2) from twin 1 born to patient 3; lanes 6 and 7, ear (strain 3NB2-1) and umbilicus (strain 3NB2-2) samples from twin 2 born to patient 3; lane 8, vaginal sample from patient 4 (strain 4M); lanes 9 to 13, ear sample (strain 4NB-2), gastric aspirate (strain 4NB-1), umbilicus sample (strain 4NB-5), right eye sample (strain 4NB-3), and left eye sample (strain 4NB-4) from a neonate born to patient 4. Neonate strains 3NB2-2, 4NB-3, 4NB-4, and 4NB-5 were isolated 5 days after strains 3M and 4M from their respective mothers.

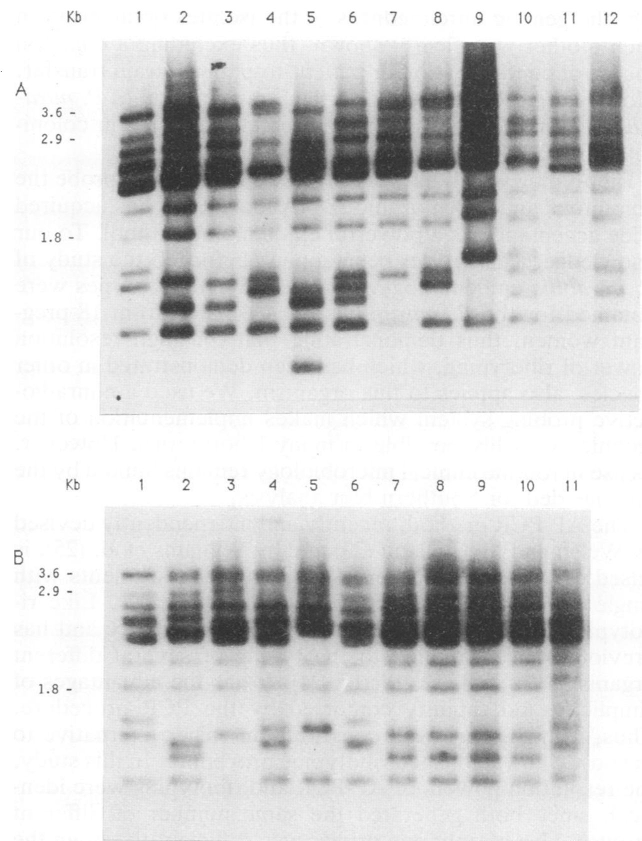

FIG. 2. *P. mirabilis* rDNA RFLP ribotyping patterns obtained after *Hin*dIII digestion. (A) Lane 1, strain ATCC 29906; lanes 2 to 12, isolates from 11 of the 18 pregnant women. (B) Lane 1, strain ATCC 29906; lanes 2 to 7 and 11, isolates from the seven remaining pregnant women; lane 7, vaginal sample (strain 3M) from patient 3; lanes 8 and 9, ear sample (strain 3NB1-1) and gastric aspirate (strain 3NB1-2) from twin 1 born to patient 3; lane 10, ear sample (strain 3NB2-1) from twin 2 born to patient 3.

for 7% of cases of postpartum endometritis (16). However, the sudden temporal clustering of cases of vaginal colonization with *P. mirabilis* has been a cause of alarm in our maternity. Indeed, the birth canal is considered the primary site where the neonate may acquire organisms and thus become colonized or infected. Infections caused by *P. mirabilis* are frequently complicated by brain abscesses with a poor outcome and high mortality rates (15). Thus, *P. mirabilis* must be considered a potential pathogen for neonates. Efficient measures for the prevention of *P. mirabilis* colonization depend on our ability to identify the source of contamination and to accurately differentiate independent from related strains.

Up to now, epidemiological studies of *P. mirabilis* have been based essentially upon the study of phenotypic traits. Although such studies are very useful, there are some problems associated with these techniques. Biochemical profiles and antibiotic sensitivity patterns do not reliably show enough strain-to-strain variations to be sufficiently discriminatory. Serotyping of *P. mirabilis* is useful for characterizing strains, but some combinations of O and H antigens are common, and thus, some strains require additional typing procedures (20). The Dienes test is simple and rapid and can be used for the detection of cross infection (18, 21). However, some investigators, by testing isolates randomly, found the test unreliable (7, 10). Typing methods based upon susceptibility to bacteriophage lysis or proticin production and sensitivity require availability of specific reagents. Recently, multilocus enzyme electrophoresis analysis has been used for the epidemiology of *P. mirabilis* (11). However, this approach may be impractical in many clinical laboratories because the procedure is complex, labor-intensive, and time-consuming and it requires a considerable amount of standardization. Genetic analysis of plasmids is simple to perform and has often been used as an epidemiological tool. However, plasmid analysis with or without further characterization by restriction endonuclease cleavage has the obvious disadvantage of being limited to plasmid-containing strains and by the fact that plasmids are unstable and transposable genetic elements.

We used genomic DNA fingerprinting by AP-PCR and ribotyping for the epidemiological evaluation of our *P. mirabilis* isolates. On the basis of phenotypic characteristics such as the biochemical profile and the susceptibility resistance pattern, our conclusion would have been that the occurrence of this *P. mirabilis* outbreak was the result of the spread of seven epidemiological strains in the maternity. In contrast, on the basis of the results of AP-PCR and ribotyp-

ing, the genetic unrelatedness of the isolates obtained from each mother was clearly shown, thus excluding a common source of contamination or patient-to-patient strain transfer. Moreover mother-to-infant vertical transmission of *P. mirabilis* occurred in four cases, fortunately resulting in colonization only.

Ribotyping which uses labelled *E. coli* rRNA to probe the ubiquitous and polymorphic rDNA loci (9, 22) has acquired wide acceptance as a powerful epidemiological tool. To our knowledge, it has never been applied before to the study of *P. mirabilis* epidemiology. In our study, 18 ribotypes were obtained for the *P. mirabilis* strains isolated from 18 pregnant women, thus demonstrating that the high resolution power of ribotyping, which has been demonstrated in other species, also applies to this organism. We used a nonradioactive probing system which makes implementation of the technique readily possible in many laboratories. However, its use in routine clinical microbiology remains limited by the time needed for Southern blot analysis.

The AP-PCR method, recently and independently devised by Welsh and McClelland (23) and by Williams et al. (25), is based on the amplification of random DNA segments with single primers of arbitrary nucleotide sequences. Like ribotyping, it is a method with general applicability and has previously been applied to the typing of several different organisms (1, 2, 8, 12, 14, 17, 24). It has the advantages of simplicity and rapidity conferred by the PCR procedure. Thus, it might very well constitute an easier alternative to the now well-established ribotyping procedure. In this study, the resolution powers of AP-PCR and ribotyping were identical, since both generated the same number of different profiles. Use of only one primer was sufficient to obtain the desired level of discrimination, since strains for each mother produced a distinct profile. Others have shown that increasing the number of primers increases the discrimination of AP-PCR (8), just as increasing the number of restriction endonucleases increases the discrimination of ribotyping. Although the reproducibility and stability of AP-PCR are suggested by several reports (12, 14), these issues have not been fully addressed yet. Here, the reproducibility of the method is clear from the results shown in Fig. 1B. Indeed, in this figure, lanes 3 to 7 and 8 to 13, which each correspond to a different mother-newborn pair, show a strict homogeneity of the patterns for each pair even though DNAs were independently prepared from strains isolated from different sites in the newborns. Similarly, a good indication of the stability of AP-PCR markers in vivo comes from the fact that some strains of the newborns were isolated 5 days after delivery, i.e., the date of isolation of the initial strains.

No potential cause of this sudden clustering of *P. mirabilis* maternal carriages has been identified yet. *P. mirabilis* is commonly present in the normal intestinal flora, which has been shown to be a source of extraintestinal infections (6, 7). It is possible that in our patients some specific, but as-yet-unidentified, cause has favored the movement of *P. mirabilis* from the intestinal tract to the vagina. This would account for the complete unrelatedness of the strains isolated from the pregnant women.

In conclusion, we find that AP-PCR is a rapid method which seems particularly well suited to the epidemiological study of *P. mirabilis* isolates.

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REFERENCES

1. **Baranton, G., D. Postic, I. Saint Girons, P. Boerlin, J.-C. Piffaretti, M. Assous, and P. A. D. Grimont.** 1992. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. Int. J. Syst. Bacteriol. **42:**378–383.
2. **Bingen, E., H. Cavé, Y. Aujard, N. Lambert-Zechovsky, P. Desjardins, J. Elion, and E. Denamur.** 1993. Molecular analysis of multiply recurrent meningitis due to *Escherichia coli* K1 in an infant. Clin. Infect. Dis., **16:**82–85.
3. **Bingen, E., E. Denamur, N. Lambert-Zechovsky, Y. Aujard, N. Brahimi, P. Geslin, and J. Elion.** 1992. Analysis of DNA restriction fragment length polymorphism extends the evidence for breast milk transmission in *Streptococcus agalactiae* late-onset neonatal infection. J. Infect. Dis. **165:**569–573.
4. **Bingen, E. H., E. Denamur, N. Y. Lambert-Zechovsky, A. Bourdois, P. Mariani-Kurkdjian, J.-P. Cezard, J. Navarro, and J. Elion.** 1991. DNA restriction fragment length polymorphism differentiates crossed from independent infections in nosocomial *Xanthomonas maltophilia* bacteremia. J. Clin. Microbiol. **29:**1348–1350.
5. **Burke, J. P., D. Ingall, J. O. Klein, H. M. Gezon, and M. Finland.** 1971. *Proteus mirabilis* infections in a hospital nursery traced to a human carrier. N. Engl. J. Med. **284:**115–121.
6. **Chow, A. W., P. R. Taylor, T. T. Yoshikawa, and L. B. Guze.** 1979. A nosocomial outbreak of infections due to multiply resistant *Proteus mirabilis*: role of intestinal colonization as a major reservoir. J. Infect. Dis. **139:**621–627.
7. **DeLouvois, J.** 1969. Serotyping and the Dienes reaction on *Proteus mirabilis* from hospital infections. J. Clin. Pathol. **22:**263–268.
8. **Fekete, A., J. A. Bantle, S. M. Halling, and R. W. Stich.** 1992. Amplification fragment length polymorphism in *Brucella* strains by use of polymerase chain reaction with arbitrary primers. J. Bacteriol. **174:**7778–7783.
9. **Grimont, F., and P. A. D. Grimont.** 1986. Ribosomal ribonucleic acid gene restriction patterns as potential taxonomic tools. Ann. Inst. Pasteur Microbiol. **137B:**165–175.
10. **Hickman, F. W., and J. J. Farmer III.** 1976. Differentiation of *Proteus mirabilis* by bacteriophage typing and the Dienes reaction. J. Clin. Microbiol. **3:**350–358.
11. **Kappos, T., M. A. John, Z. Hussain, and M. A. Valvano.** 1992. Outer membrane protein profiles and multilocus enzyme electrophoresis analysis for differentiation of clinical isolates of *Proteus mirabilis* and *Proteus vulgaris*. J. Clin. Microbiol. **30:**2632–2637.
12. **Kersulyte, D., J. P. Woods, E. J. Keath, W. E. Goldman, and D. E. Berg.** 1992. Diversity among clinical isolates of *Histoplasma capsulatum* detected by polymerase chain reaction with arbitrary primers. J. Bacteriol. **174:**7075–7079.
13. **Kotelko, K.** 1986. *Proteus mirabilis*: taxonomic position, peculiarities of growth, components of the cell envelope. Curr. Top. Microbiol. Immunol. **129:**181–215.
14. **Lehmann, P. F., D. Lin, and B. A. Lasker.** 1992. Genotypic identification and characterization of species and strains within the genus *Candida* by using random amplified polymorphic DNA. J. Clin. Microbiol. **30:**3249–3254.
15. **Levy, H. L., and D. Ingall.** 1967. Meningitis in neonates due to *Proteus mirabilis*. Am. J. Dis. Child. **114:**320–324.
16. **Martens, M. G., S. Faro, M. Maccato, G. Riddle, and H. A. Hammill.** 1991. Susceptibility of female pelvic pathogens to oral antibiotic agents in patients who develop post-partum endometritis. Am. J. Obstet. Gynecol. **164:**1383–1386.
17. **Mazurier, S. I., and K. Wernars.** 1992. Typing of *Listeria* strains by random amplification of polymorphic DNA. Res. Microbiol. **143:**499–505.
18. **Penner, J. L.** 1981. The tribe Proteeae, p. 1204–1224. *In* M. P. Starr (ed.), The prokaryotes: handbook on habitats, isolation and identification of bacteria. Springer-Verlag, New York.
19. **Senior, B. W.** 1977. The Dienes phenomenon: identification of the determinants of compatibility. J. Gen. Microbiol. **102:**235–244.
20. **Senior, B. W., and P. Larsson.** 1983. A highly discriminatory multi-typing scheme for *Proteus mirabilis* and *Proteus vulgaris*.

J. Med. Microbiol. **16:**193–202.
21. **Skirrow, M. B.** 1969. The Dienes (mutual inhibition) test in the investigation of *Proteus mirabilis*. J. Med. Microbiol. **2:**471–477.
22. **Stull, T. L., J. J. Lipuma, and T. D. Edlind.** 1988. A broad spectrum probe for molecular epidemiology of bacteria: ribosomal RNA. J. Infect. Dis. **157:**280–286.
23. **Welsh, J., and M. McClelland.** 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. **18:**7213–7218.
24. **Welsh, J., C. Pretzman, D. Postic, I. Saint Girons, G. Baranton, and M. McClelland.** 1992. Genomic fingerprinting by arbitrarily primed polymerase chain reaction resolves *Borrelia burgdorferi* into three distinct phyletic groups. Int. J. Syst. Bacteriol. **42:**370–377.
25. **Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey.** 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18:**6531–6535.