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Genomic Fingerprinting of Epidemic and Endemic Strains of *Stenotrophomonas maltophilia* (Formerly *Xanthomonas maltophilia*) by Arbitrarily Primed PCR

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Arbitrarily primed PCR (AP-PCR) was used to type 64 clinical isolates of *Stenotrophomonas maltophilia* from 60 patients and the hands of one nurse. Forty-seven different patterns were observed, most patients having isolates with unique genomic fingerprints. A single pattern, however, was obtained from six of eight patients involved in an intensive care nursery outbreak, confirming the suspected nosocomial transmission of this microorganism. This strain was also found in four other patients hospitalized at the same time but in different units. AP-PCR typing results had a good correlation with the 49 patterns obtained when the isolates were typed by contour-clamped homogeneous electric field gel electrophoresis. Although AP-PCR is slightly less discriminatory than contour-clamped homogeneous electric field gel electrophoresis, it offers several advantages and should be considered as a practical option for molecular typing during investigations of outbreaks.

Stenotrophomonas maltophilia, formerly known as *Xanthomonas maltophilia* (9), is an increasingly important nosocomial pathogen in hospitalized patients. It is resistant to most commonly tested antibiotics and so presents a challenge for treatment. Strategies for its containment may best be found in preventive measures. Identifying the source of the patient's bacterial infection is often difficult because the organism is ubiquitous in the environment. Thus, when infections occur in the hospital setting, it is unclear how many are independently acquired compared with the number resulting from cross-transmission. To enhance traditionally obtained epidemiological data, a genotyping technique capable of discriminating individual strains of the organism is required. We have previously demonstrated that contour-clamped homogeneous electric field gel electrophoresis (CHEF) has a powerful ability to discriminate between strains of *S. maltophilia* (12). One limitation of CHEF is that it takes several days to complete, which can be of significance when conducting an investigation of an outbreak. It also requires the use of specialized equipment not available in many laboratories.

The PCR is a simple and rapid technique which has recently been used to detect genomic polymorphism at the strain level by using a single arbitrary primer in a reaction referred to as arbitrarily primed PCR (AP-PCR) or random amplified polymorphic DNA-PCR (14, 15). This technique has been used to genotype a variety of microorganisms in epidemiological investigations (1–3, 6). There is a need to validate the reproducibility and applicability of this appealing technique, as well as to compare its discriminatory power with those of other molecular techniques.

AP-PCR has several theoretical advantages over CHEF. It can be completed in a single day and involves technology familiar to most molecular laboratories. Because primers can be selected without regard to known sequences, a laboratory could stock a limited number of primers and have the capability of strain typing a variety of microorganisms. The purpose of this study was to determine if epidemiologically related and unrelated strains of *S. maltophilia* infection could be rapidly and accurately typed with AP-PCR. We have characterized 64 clinical isolates with AP-PCR and compared these results with those obtained previously by CHEF (12).

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MATERIALS AND METHODS

Bacterial isolates. Sixty-four isolates were obtained from 60 patients and from the hands of one nurse over a 9-month period. Eight of the patients and the nurse were involved in an outbreak of *S. maltophilia* infection in an intensive care nursery.

AP-PCR methods. DNA was obtained as previously described (11). Briefly, isolated colonies were scraped from culture plates and boiled in TE (1 mM Tris, 1 mM EDTA [pH 8.0]) for 10 min and then had particulates pelleted by centrifugation at 2,500 × *g* for 3 min. DNA in the supernatant was quantitated by spectrophotometry at an optical density of 260 nm. PCR mixtures were prepared in 100 μl of 1× buffer (50 mM KCl, 20 mM Tris-HCl, 2.5 mM $MgCl_2$, 100 μg of bovine serum albumin per ml [pH 8.4]) and contained 1 μg of DNA, 0.1 mM (each) deoxynucleoside triphosphate, 2 U of recombinant thermostable DNA polymerase (r*Taq*; Perkin-Elmer Cetus, Norwalk, Conn.), and 30 pmol of arbitrarily chosen primer (pBR322 *Sal*I site primer counterclockwise [AGTCATGC CCCGCGC]). In preliminary studies, this G+C-rich primer was found to produce a better banding pattern than primers with lower G+C content (37 to 53%), which resulted in either no amplification, amplification of fewer bands, or poor reproducibility (13). Each reaction mixture was overlaid with 150 μl of mineral oil. PCR was initiated with five cycles of low stringency, which included a denaturing step at 95°C for 1 min, annealing of the primer at 28°C for 1 min, and 2 min of extension at 72°C. After the initial 5 cycles, 55 additional cycles were conducted with annealing of the primer at 50°C. The reaction was terminated with a final extension cycle at 72°C for 10 min. To ensure reproducibility, PCRs were carried out with DNA from duplicate colonies and in duplicate with DNA from one of the preparations. Thus, each isolate was represented by three reactions. Selected isolates were regrown from freezer stock and retested on another day.

Visualization of amplified products. Amplification products (12 μl) were electrophoresed in a 1.5% agarose gel (15 by 9 cm) in 1× Tris-borate-EDTA buffer for 90 min at 100 V and visualized under UV light after staining with ethidium bromide. Multiple gels were run so that each isolate could be compared against each other isolate on the same gel.

CHEF methods. The CHEF methods have been previously described (12). Briefly, agarose plugs containing *Xba*I- or *Ssp*I-digested chromosomal DNA were electrophoresed in 1.2% Fast Lane agarose (FMC Bioproducts, Rockland, Maine) gels in 0.5× Tris-acetate-EDTA for 20 h at 6 V/cm. Ramp conditions were 5 to 35 s for *Xba*I digests and 5 to 25 s for *Ssp*I digests.

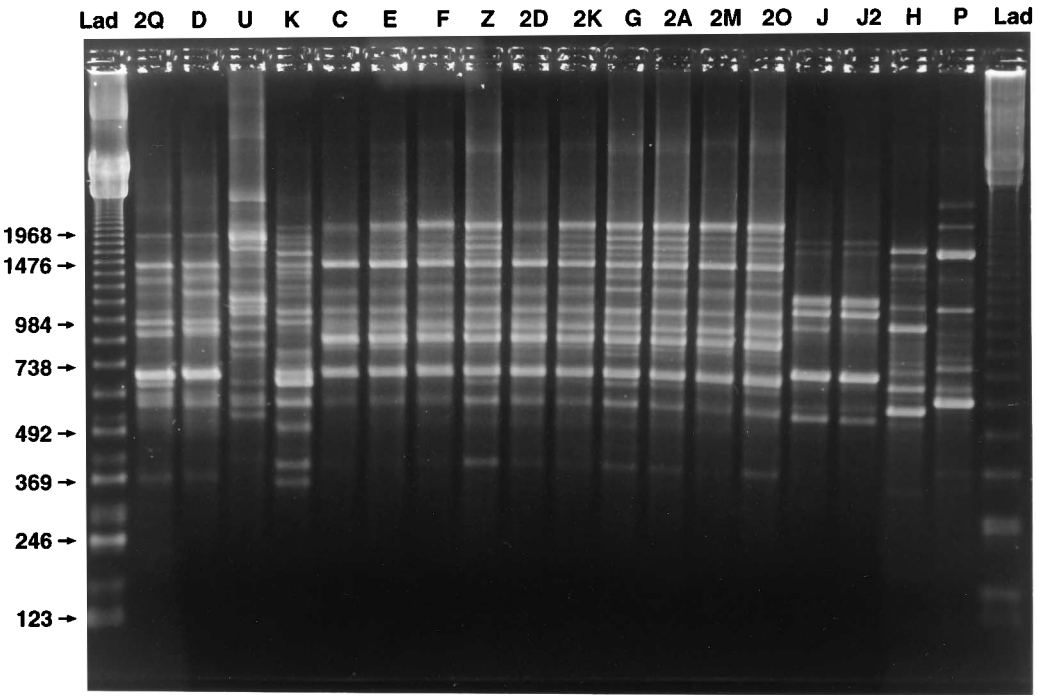

FIG. 1. AP-PCR patterns of *S. maltophilia* isolates. Isolates 2Q and D had similar patterns despite no clear epidemiological link between the patients. D, U, and K were all obtained from the outbreak in the intensive care nursery but did not match the outbreak pattern of the other six intensive care nursery patients (C, E, F, Z, 2D, and 2K). The outbreak pattern was identified in four other patients (G, 2A, 2M, and 2O) hospitalized during the outbreak but in different units. Patients J and G had different patterns despite being hospitalized in nearly adjacent rooms in the burn unit. Sputum (J) and blood (J2) isolates from the same patient matched. Other unrelated isolates (H and P) had unique patterns. The outside lanes are 123-bp ladders (Lad).

Comparison of AP-PCR and CHEF results. Patterns of AP-PCR amplification products for each isolate were initially analyzed and compared by one of us (C.V.) who was familiar with CHEF results. For two isolates to be considered the same, the pattern had to match without regard to band intensity and without a requirement that there be a band to match a weak band in another isolate. Two investigators (Y.T. and S.C.) blinded to CHEF results compared 54 randomly selected isolates on three gels by the same criteria. This represented a total of 486 comparisons between pairs of isolates. When the same isolate was selected more than once for the same gel, an attempt was made to use samples from different PCR mixtures.

RESULTS

Sixty-four *S. maltophilia* isolates from 60 patients and one nurse were strain typed by AP-PCR. Representative isolates are shown in Fig. 1. These isolates and patients have been previously described (12). Forty-seven DNA banding patterns were observed (Table 1). Isolates obtained from six of the eight intensive care nursery patients and from four other patients who were hospitalized at the same time showed a highly related AP-PCR pattern (group I). As expected, isolates which were obtained from different sites on the same patient were indistinguishable in all three cases (groups II, III, and IV). Group VI was represented by three isolates; groups V, VII, and VIII were represented by two isolates each. In two of these cases (groups V and VIII), the patients within the group were hospitalized in the same unit at the same time. The remaining 39 isolates showed unique DNA banding patterns. The isolate obtained from the intensive care nursery nurse was unique. Unfortunately, the isolate obtained from the infant this nurse had been caring for was lost, so we were not able to compare the isolates. For three of the isolates, it appeared that two mutually exclusive patterns could be obtained for each (data not shown). Two of these isolates were classified in group V, and both patterns were shared. The third isolate produced two unique patterns. AP-PCRs from these isolates were run multiple times. The ability of each isolate to produce two specific yet different patterns was reproducible and occurred even when the reactions were run from the same DNA preparation of a single colony. Correlations were found between AP-PCR typing and the CHEF results, with the exception of the three isolates in AP-PCR group VI, which were found by CHEF to be a clonally related pair (D and 2Q) and a unique isolate (Q), and the two isolates in AP-PCR group VIII, which were found by CHEF to be unique. DNA banding patterns were reproducible, except for variations in band intensities or loss of weak bands, which occurred in approximately one of every six reactions. When these were seen, a DNA banding pattern was selected for the isolate on the basis of the most complex yet

TABLE 1. Molecular profiles of *S. maltophilia* isolates generated by AP-PCR and by CHEF

Isolate(s)	Group identification by:	
	AP-PCR	CHEF
C, E, F, G, Z, 2A, 2D, 2K, 2M, 2O	I	I
J, J2	II	II
2E, 2E2	III	III
2F, 2F2	IV	IV
2Z, 3D	V	V
D	VI	VIa
2Q	VI	VIb
Q	VI	Unique[a]
N, 3G	VII	VII
S, T	VIII	Unique
Other 39	Unique	Unique

[a] Unique, unique DNA banding patterns for each isolate.

reproducible pattern observed when multiple samples were run. This type of pattern was used for the comparisons with the other isolates.

In 486 paired comparisons between isolates, the two blinded reviewers agreed with the findings of the nonblinded reviewer 481 and 485 times, respectively. In no instance were isolates determined to be alike which the nonblinded reviewer had found to be different. The only pair of similar isolates missed by both blinded reviewers was a case in which one reaction of a duplicate run failed to show a weak band which was seen in the other reaction.

DISCUSSION

AP-PCR is a rapid and reproducible method of typing *S. maltophilia*. Forty-seven unique DNA banding patterns were observed from 64 clinical isolates. Duplicate reactions of each isolate from the same or different DNA preparations generally gave highly reproducible results.

In this study, we selected a 15-mer primer containing 73% G+C bases, which closely matches the G+C content of the *Stenotrophomonas* genome. DNA banding patterns were polymorphic between isolates, with the number of bands ranging from 3 to 15. Primers with lower G+C content did not amplify well, probably because of the high number of mismatches or the lower level of stabilization created by the double hydrogen bond of the A═T pairing compared with the triple bond of the C≡G pairing.

The G+C-rich primer used in this study generated a relatively complex pattern containing both strong and weak bands. We found in some cases that the intensity of bands varied between duplicate amplifications of DNA from the same isolate even when compared on the same gel to avoid missing bands due to variations in staining intensity or photographic quality. This is consistent with the experience of others using this typing technique (2, 7, 8). At least two factors may account for this. Strong bands may represent amplification of repetitive sequences or amplification of different products which coincidentally have the same size. Weak bands may represent a higher level of mismatch between the primer and the template, allowing amplification off one template but not off other identical templates present in the mixture in any single cycle. Variation in band intensity and failure to detect previously amplified weak bands made comparisons between AP-PCR banding patterns difficult in some cases. An interesting finding was the presence of the two mutually exclusive patterns seen for each of three isolates. The significance of this finding is unknown at this time, but it raises concern about the use of this method to type strains when a single reaction is used to compare strains. We recommend running reactions in triplicate to reduce the possibility of missing similar strains.

AP-PCR conditions such as annealing temperature, template and primer concentrations, Mg^{2+} concentration, or primer length can change the number of DNA fragments amplified for any given isolate (1, 5, 6). Adjusting these parameters may be an approach to reduce the number of weak bands seen after PCR. However, there may be loss of discriminatory power, because some strong bands are shared by almost all strains (2, 7, 11). Some investigators have used more than one primer in a multistep AP-PCR approach to differentiate between strains which appear to be the same with the use of only a single primer (8, 10). Because we found most of the weak bands were reproducible, we chose to consider them and were able with a single primer to type *S. maltophilia* strains with nearly the discriminatory power we previously demonstrated with CHEF.

The increase in typing sensitivity of CHEF over AP-PCR has also been reported by others. By summing the results of AP-PCR profiles generated with three different primers, Saulnier et al. (10) were able to identify 25 AP-PCR patterns in a group of 26 strains of *Staphylococcus aureus* shown to be different by CHEF. Bingen et al. (4) used AP-PCR to identify four different strains of *Pseudomonas cepacia* in a group of 23 isolates, whereas CHEF was able to discriminate five strains. Similarly, in our study, the improvement in sensitivity of CHEF over AP-PCR was a small one, identifying 49 CHEF patterns in a group of isolates having 47 AP-PCR patterns. Notwithstanding the difference in sensitivity, however, there are practical advantages to AP-PCR. Under the conditions outlined in this paper and given time for same-gel comparisons of duplicate reactions and then for comparisons between type patterns of isolates, AP-PCR can realistically be considered a 24-h procedure, whereas CHEF takes 4 days to complete. Thus, even though it is generally not as discriminatory as CHEF, there remains a viable and practical role for AP-PCR in epidemiological workups.

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