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Molecular Typing of *Stenotrophomonas* (*Xanthomonas*) *maltophilia* by DNA Macrorestriction Analysis and Random Amplified Polymorphic DNA Analysis

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***Stenotrophomonas* (*Xanthomonas*) *maltophilia* is a multidrug-resistant, nosocomial pathogen for which optimal typing methods in epidemiologic investigations of nosocomial outbreaks have not been defined. We compared DNA macrorestriction analysis by pulsed-field gel electrophoresis (PFGE) with random amplified polymorphic DNA (RAPD) analysis by arbitrarily primed PCR for molecular typing of 109 multidrug-resistant strains of *S. maltophilia* from multiple outbreaks at our institution over a 10-month period in 1993. PFGE after digestion with restriction endonuclease *Dra*I revealed 62 unique DNA restriction profiles among the 109 strains, with 23, 11, 6, 6, and 3 strains having concordant profiles in each of five types. There were four concordant profiles among 8 strains (2 strains with each profile), while unique profiles were present in each of the remaining 52 strains. Further RAPD analysis with a decanucleotide primer showed the same number of distinct strain types as PFGE but more subtype diversity within each clonal type. We concluded that DNA macrorestriction analysis and RAPD analysis are sufficiently discriminatory and useful for differentiation of *S. maltophilia* strains in epidemiologic investigations of nosocomial outbreaks. However, RAPD analysis by arbitrarily primed PCR is faster and less laborious method of molecular typing.**

Stenotrophomonas maltophilia, previously called *Xanthomonas maltophilia* (8), is an important nosocomial pathogen causing multidrug-resistant infections in hospitalized patients (2, 5–7). Little is known about the epidemiology of this pathogen within hospital environments, in part because of the lack of a reliable method for typing of clinical isolates in epidemiologic investigations of nosocomial outbreaks due to *S. maltophilia*. Antibiogram profiles are unreliable for typing since these isolates are frequently resistant to multiple antibiotics. Other typing methods, such as serologic typing (11), multilocus enzyme electrophoresis (12), and DNA restriction endonuclease analysis (1), have been developed, but the former two are not readily available or easily adaptable in many clinical microbiology laboratories whereas the latter generates DNA restriction profiles that are difficult to interpret. Recently, pulsed-field gel electrophoresis (PFGE) of genomic DNA (4) and arbitrarily primed PCR (AP-PCR) (13) have been developed as reliable molecular typing methods for epidemiologic investigations of outbreaks due to various nosocomial pathogens. A recent outbreak of *S. maltophilia* colonization and infections among patients at our hospital provided an opportunity to apply molecular methods for epidemiologic typing of clinical isolates. The purpose of the present study was to compare DNA macrorestriction analysis by PFGE with random amplified polymorphic DNA (RAPD) analysis by AP-PCR for molecular typing of clinical isolates of *S. maltophilia* in an epidemiologic investigation of a nosocomial outbreak.

Strains of *S. maltophilia* chosen for study were consecutively isolated from clinical specimens submitted to the Clinical Microbiology Laboratory of the The Toronto Hospital, Toronto, Ontario, Canada, during the first 10 months of 1993. Isolates were identified initially by typical colony morphology, Gram stain appearance, and the Negative Combo panel type 15 of the MicroScan identification-susceptibility system (WalkAway-96; Baxter Healthcare Corp., Mississauga, Ontario, Canada). Identification was confirmed by results of conventional biochemical tests: weak oxidase reaction and positive reactions for gelatin hydrolysis, DNase, esculin hydrolysis, and lysine decarboxylase (3). *S. maltophilia* ATCC 13637 was used as a reference strain for comparison.

Strains were grown overnight in 3-ml volumes of brain heart infusion (Difco Laboratories, Detroit, Mich.) broth. After centrifugation at 3,000 × *g* for 10 min, each cell pellet was suspended in 1 ml of SE buffer (25 mM EDTA [pH 7.4], 75 mM NaCl). Agarose plugs were made from a 1:1 mixture of 1.6% low-melting-point agarose (Bio-Rad, Richmond, Calif.) and the cell suspension. Each plug was placed in 1.5 ml of lysis buffer (10 mM Tris-HCl [pH 7.6], 100 mM EDTA [pH 8.0], 50 mM NaCl, 0.2% deoxycholic acid [Sigma Chemical Co., St. Louis, Mo.], 1% *N*-lauroyl sarcosine [Sigma], 2 mg of lysozyme [Sigma] per ml) for 3 h at 35°C. Samples were then treated for 16 h at 42°C with the same volume of a proteinase K solution containing 50 μg of proteinase K (Boehringer Mannheim, Laval, Quebec, Canada) per ml, 100 mM EDTA (pH 8.0), 0.2% deoxycholic acid, and 1% *N*-lauroyl sarcosine. After three 1-h washes with TE buffer (10 mM Tris-HCl, 0.1 mM EDTA [pH 8.0]), the agarose plugs were stored in TE buffer at 4°C for subsequent PFGE.

Agarose plugs were digested with restriction enzyme *Dra*I (Pharmacia P-L Biochemicals, Milwaukee, Wis.) for 20 h at 35°C in accordance with the manufacturer's recommendations. The resultant DNA fragments were separated on a 1% PFGE agarose (Bio-Rad) gel in a contour-clamped homogeneous electrical field by using the CHEF DR-II system (Bio-Rad) with 0.5× TBE buffer (45 mM Tris-borate, 1 mM EDTA [pH 8.0]) at 12°C. With the voltage set at V/cm, pulse times ranged from 5 to 45 s over 20 h with linear ramping. The procedure

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was repeated at least once for each isolate to determine the reproducibility of the results.

After an overnight culture on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.), a 10-μl volume of cells was scraped from the agar plate with a calibrated inoculation loop and washed in 300 μl of sterile saline. Cells were resuspended in 300 μl of distilled water and heated at 100°C for 10 min. The boiled suspensions were kept at 4°C for later use as target DNA templates for RAPD analysis. Each 25-μl reaction mixture contained 0.025 U of *Taq* polymerase (AmpliTaq; Roche Diagnostics, Branchburg, N.J.) per μl; dATP, dGTP, dTTP, and dCTP at 200 mM each (Boehringer Mannheim); and 1 μM primer A (5′-CTGGCGGCTG-3′) or B (5′-CAG GCGGCGT-3′) (DNA Synthesis Facility, Biotechnology Service Centre, Hospital for Sick Children, Toronto, Ontario, Canada) in PCR buffer (100 mM Tris-HCl [pH 8.5], 50 mM KCl, 4 mM $MgCl_2$). After an initial 2 min of denaturation at 94°C, reaction mixtures were run through 35 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 35°C, and extension for 1 min at 72°C. A final extension was carried out for 4 min at 72°C. Samples were electrophoresed for 3 h at 90 V on 1.5% agarose (SeaKem LE; FMC BioProducts, Rockland, Maine) containing 0.5 μg of ethidium bromide per ml in 1× TAE buffer (40 M Tris-acetate, 1 mM EDTA [pH 8.0]). Reproducibility of results was examined by repeating the AP-PCR procedure twice for each isolate.

DNA profiles generated by PFGE and RAPD were compared visually and analyzed for relatedness in accordance with the Goering criteria (4). Isolates were considered to represent the same strain if their DNA profiles were indistinguishable, whereas isolates were categorized as unrelated strains when their DNA profiles differed by more than three DNA band shifts. Isolates with DNA profiles differing by three or fewer DNA band shifts were deemed related strains (i.e., subtypes of a strain), as each DNA band shift represents a single genetic event.

During the 10-month period from January to October 1993, 109 nonduplicate strains of *S. maltophilia* were isolated from patients at our institution. The sources of these isolates were 33 sputum samples (30%) 24 endotracheal aspirates (22%), 13 bronchoalveolar lavage fluid samples (12%), 11 wounds (10%), 8 urine samples (7%), 5 blood samples (5%), and 15 samples from other body sites (14%). PFGE after restriction by *Dra*I endonuclease generated discernible DNA macrorestriction profiles of 8 to 18 DNA bands in each (Fig. 1). RAPD analysis with primer A produced DNA profiles containing 8 to 20 DNA bands, while RAPD anlaysis with primer B yielded 8 to 18 DNA bands in each (Fig. 2). Sixty-two unique DNA profiles were obtained by PFGE and RAPD analysis among the 109 clinical isolates. There was good correlation of the DNA profiles between PFGE and RAPD analysis, as shown in Table 1. Only one DNA profile (type 11) obtained by PFGE was found to have two strain subtypes. However, up to three strain subtypes of a DNA profile may be produced by RAPD analysis for a given DNA macrorestriction profile by PFGE. Both primers A and B yielded the same number of unique DNA profiles.

Identical results were obtained by repeated runs of PFGE with all of the isolates, with consistent DNA profiles. RAPD analysis generated DNA profiles in which two to four DNA bands may vary considerably in intensity from one run to another, despite use of the same reaction conditions and the same primer (data not shown).

Of the 109 *S. maltophilia* strains studied, 83 (76%) were considered to be of nosocomial origin and were isolated from patients at >72 h after hospitalization. Among these 83 isolates, 52 and 31 isolates were from patients in intensive care units and wards, respectively. DNA macrorestriction profile types 1 and 2 accounted for 32 (61.5%) of the 52 isolates from the intensive care units but only 2 of the 31 ward isolates. Similar correlations were observed with RAPD analysis profile types 1 and 2. In contrast, 26 different profiles were found among the remaining 29 ward isolates by DNA macrorestriction and RAPD analyses. Within a geographically confined and epidemiologically linked setting in the hospital, i.e., intensive care units, the clustering of isolates with two unique macrorestriction profiles strongly suggests nosocomial transmission of these strains from either a common source and/or between patients.

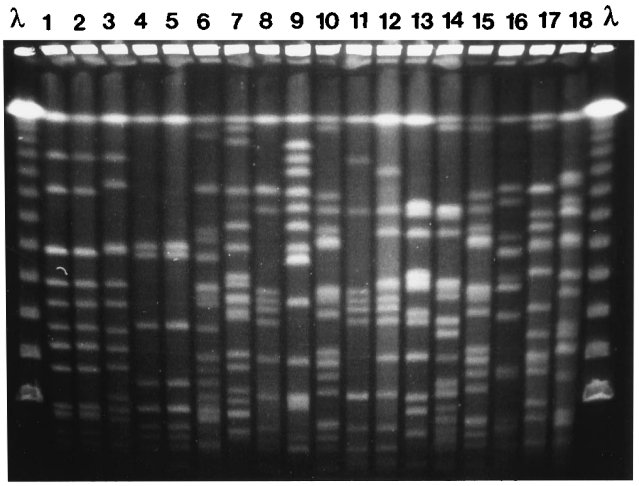

FIG. 1. Genomic DNA macrorestriction profiles of *S. maltophilia* produced by PFGE after *Dra*I digestion. Hospital ward locations of isolates are as follows: surgical intensive care unit, lanes 1, 2, 4, 5, 6, and 13; medical intensive care unit, lanes 3, 7, 9, 10, and 17; individual wards, lanes 12, 14, and 15; outpatient clinic, lanes 8, 11, and 16. Lane 18, *S. maltophilia* ATCC 13637. λ, lambda ladder marker (size range, 50 to 1,000 kb).

The outbreak of *S. maltophilia* among patients at our hospital provided an opportunity to compare the abilities of DNA macrorestriction analysis and RAPD analysis to differentiate clinical isolates of *S. maltophilia* for molecular epidemiologic investigation of a nosocomial outbreak. Both PFGE with *Dra*I restriction and AP-PCR with primers A and B yielded DNA profiles with suitable numbers of DNA bands for differentiation of the 109 clinical isolates examined. Our results corroborate the findings of Sader et al. (10), who found that DNA macrorestriction analysis by PFGE after *Xba*I digestion produced discriminatory, reproducible DNA profiles for epidemiological typing of *S. maltophilia* strains from three different countries.

Compared with the procedure of PFGE, which usually requires a minimum of 4 days to complete, RAPD analysis takes less time (1 day) and is less laborious. However, preliminary trials of RAPD analysis with several different oligonucleotide primers are necessary to determine the optimal primers and reaction conditions for generation of suitable numbers of DNA bands (between 5 and 20 bands) for easy discernibility and sufficient discrimination among the profiles. The decanucleotide primers used in our study contained 80 mol% G+C, which is optimal for amplification of genomic DNA fragments in the G+C-rich bacterium *S. maltophilia* (63 to 71 mol% G+C). These results support the findings of a recent study in which primers rich in G+C produced DNA banding patterns consistent with data obtained by PFGE (14). Although RAPD analysis offers excellent intralaboratory reproducibility, inter-

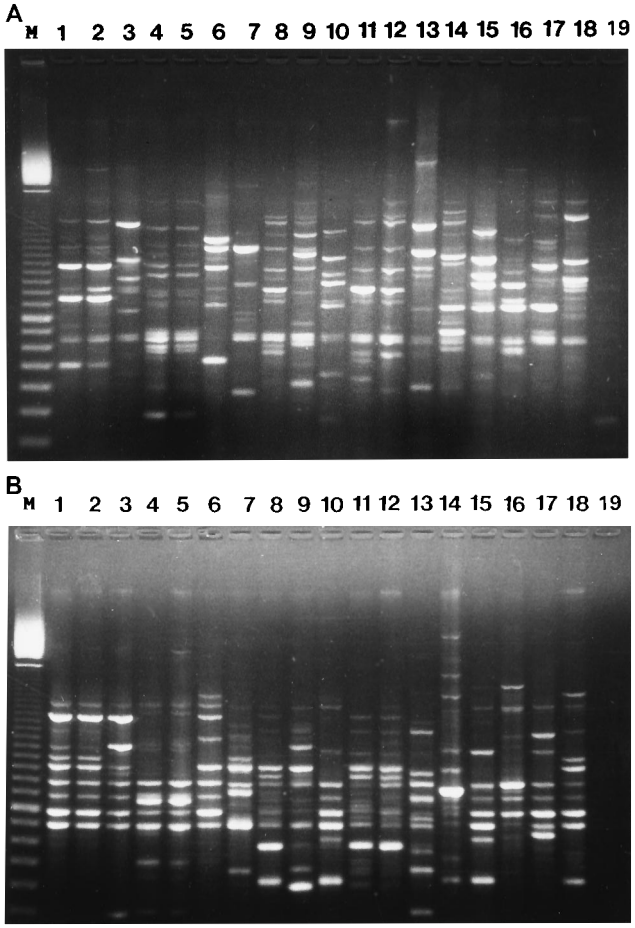

FIG. 2. DNA fingerprinting profiles after AP-PCR with primers A (A) and B (B). Hospital ward locations of isolates are as follows: surgical intensive care unit, lanes 1, 2, 4, 5, 6, and 13; medical intensive care unit, lanes 3, 7, 9, 10, and 17; individual wards, lanes 12, 14, and 15; outpatient clinic, lanes 8, 11, and 16. Lanes 18, *S. maltophilia* ATCC 13637; lanes 19, negative-reaction control (no DNA template). Lanes M, 100-bp ladder.

laboratory reproducibility is less predictable. In a recent laboratory study (9), four laboratories were unable to amplify DNA fragments provided to them by using a standardized protocol and the same set of primers. Most of the difficulties were attributed to annealing temperature differences within the different thermocyclers, as demonstrated by external thermocouple temperature readings. Reproducibility was significantly enhanced when the actual tube temperatures were standardized.

Differences in the numbers of strain subtypes obtained by PFGE and RAPD analysis likely reflect the differences in the principles of genotyping between the two methods. Use of low-frequency restriction endonucleases, such as *Dra*I, for PFGE produces DNA profiles with limited numbers of large DNA fragments for easy discernibility. However, small genomic differences among isolates would not be detectable by this procedure and less subtype diversity would be present by DNA macrorestriction analysis. In contrast, RAPD analysis yields DNA profiles with relatively small DNA fragments and the number of fragments generated depends on the genomic nucleotide sequence (13). Thus, minor differences among genomic nucleotide sequences may result in closely related, but not identical, DNA banding patterns by this typing method. RAPD analysis may be more discriminatory than DNA macrorestriction analysis for epidemiologic typing of isolates.

TABLE 1. Correlation between DNA profiles obtained by PFGE and RAPD analysis of clinical isolates of *S. maltophilia*

DNA profile type(s)[a]		No. of isolates
PFGE	RAPD analysis	
1	1 a, b, c	23
2	2 a, b, c	11
3	3 a, b	6
4	4 a, b	6
5	5	3
6–9	6–9	2[b]
10	10	1
11 a, b	11 a, b	1[b]
12–62	12–62	1[b]

[a] a, b, and c are profile subtypes.
[b] No. of isolates in each profile type.

This study of a large sample of *S. maltophilia* isolates from an institutional outbreak demonstrated the utility of PFGE and AP-PCR for epidemiologic typing of this organism, which is of increasing importance as a nosocomial pathogen. We concluded that both PFGE and RAPD analysis are useful, discriminatory DNA-based techniques with excellent intralaboratory reproducibility for differentiation of clinical isolates of *S. maltophilia*. Although RAPD analysis is a more specific, faster, and less labor-intensive method than PFGE, the former method may produce additional DNA profiles that may provide greater discrimination, but this observation should be interpreted with caution until further data are available. RAPD analysis may be used as a complementary or alternative epidemiologic typing method for *S. maltophilia*.

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