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Characterization of the Chromosomal *aac(6′)-Iz* Gene of *Stenotrophomonas maltophilia*

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The *aac(6′)-Iz* gene of *Stenotrophomonas maltophilia* BM2690 encoding an aminoglycoside 6′-*N*-acetyltransferase was characterized. The gene was identified as a coding sequence of 462 bp corresponding to a protein with a calculated mass of 16,506 Da, a value in good agreement with that of ca. 16,000 found by in vitro coupled transcription-translation. Analysis of the deduced amino acid sequence indicated that the protein was a member of the major subfamily of aminoglycoside 6′-*N*-acetyltransferases. The enzyme conferred resistance to amikacin but not to gentamicin, indicating that it was an AAC(6′) of type I. The open reading frame upstream from the *aac(6′)-Iz* gene was homologous to the *fprA* gene of *Myxococcus xanthus* (61% identity), which encodes a putative pyridoxine (pyridoxamine) 5′-phosphate oxidase. Pulsed-field gel electrophoresis of total DNA from BM2690 and *S. maltophilia* ATTC 13637 digested with *Xba*I, *Dra*I, and *Spe*I followed by hybridization with rRNA and *aac(6′)-Iz*-specific probes indicated that the gene was located in the chromosome. The *aac(6′)-Iz* gene was detected by DNA-DNA hybridization in all 80 strains of *S. maltophilia* tested. The MICs of gentamicin against these strains of *S. maltophilia* were lower than those of amikacin, netilmicin, and tobramycin, indicating that production of AAC(6′)-Iz contributes to aminoglycoside resistance in *S. maltophilia*.

Stenotrophomonas maltophilia (formerly *Xanthomonas maltophilia*) is a free-living, ubiquitous, nonfermentative gram-negative bacillus. It is an important opportunistic nosocomial pathogen in neutropenic, immunocompromised, and debilitated patients. *S. maltophilia* is associated with a wide variety of clinical infections, including bacteremia, endocarditis, meningitis, respiratory and urinary tract infections, and wound sepsis (13). More recently, increasing chronic colonization by this bacterial species of the lower respiratory tract in patients with cystic fibrosis has been reported (2, 12).

S. maltophilia is characteristically resistant to most broad-spectrum antimicrobial agents, including aminoglycosides, carbapenems, cephalosporins, penicillins, and quinolones. These antibiotics are seldom used alone (13), and synergism in vitro has been observed for certain combinations (5, 29, 49). The resistance of *S. maltophilia* is mainly attributed to permeability barriers (8, 25, 47). For instance, growth temperature-dependent variation in susceptibility to aminoglycosides, between 30 and 37°C, could be due to changes in the conformation of the outer membrane (30, 31, 46). Efflux pumps could also play a role in multiple resistance, but this mechanism has not yet been documented in this species. In addition to intrinsic resistance by impermeability, *S. maltophilia* can inactivate antibiotics by synthesis of detoxifying enzymes. Metallo-β-lactamases and cephalosporinases have been extensively investigated (9, 26, 36), and modification of aminoglycosides by *O*-nucleotidylation and *N*-acetylation has been reported, but the responsible genes have not been identified (20, 45). Among the various aminoglycoside-modifying enzymes, the AAC(6′) family is of particular interest, since it modifies antibiotics of therapeutic importance, such as amikacin, isepamicin, gentamicin, netilmicin, and tobramycin. The sequences of 22 *aac(6′)* genes encoding type I enzymes which modify amikacin but not gentamicin have been determined. Certain genes are associated with mobile elements, including plasmids, transposons, and integron cassettes (4, 40), whereas others are species specific, such as *aac(6′)-Ic* of *Serratia marcescens* (41); *aac(6′)-Ii* of *Enterococcus faecium* (6); and *aac(6′)-Ig*, *-Ij*, *-Ik*, and *-Ir* to *-Iw* of proteolytic genomospecies of *Acinetobacter* (22, 23, 34, 35). In this work, we describe the *aac(6′)-Iz* gene indigenous to *S. maltophilia* which contributes to aminoglycoside resistance in the species.

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MATERIALS AND METHODS

Bacterial strains and plasmids. Epidemiologically unrelated clinical isolates of *S. maltophilia* from different sources, including strain BM2690 isolated from the sputum of a patient with cystic fibrosis, were used. The strains were isolated between 1992 and 1998 at the Hopital Saint Michel in Paris, France. Eight additional strains isolated from cystic fibrosis patients were from Hopital Robert Debré in Paris. *S. maltophilia* ATCC 13637 was also included in this study. Certain strains and plasmids used in this study are listed in Table 1.

Strain identification and growth conditions. Identification was performed with API 20 NE strips (bioMérieux, La-Balme-les-Grottes, France). All the isolates were resistant to imipenem. The strains were grown in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) or on Mueller-Hinton (MH) agar (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France). MH medium supplemented with 0.005% 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (IPTG) was used to detect production of β-galactosidase. MICs on MH agar containing serially twofold-diluted aminoglycosides were determined by the method of Steers et al. (42) with ca. 10^4 CFU per spot. The plates were incubated at 37°C for 18 h. The activity of 2′- and 6′-*N*-ethylnetilmicin was studied by diffusion on MH agar at 37°C with disks containing 100 μg of antibiotic.

Assay for aminoglycoside-modifying enzymes. The activity of aminoglycoside-modifying enzymes was detected in bacterial extracts by the phosphocellulose paper-binding technique (17). The reaction was allowed to proceed for 30 min at 30°C.

Genetic techniques. Transformation of *Escherichia coli* JM83 was performed as described previously (38). The antibiotics for selection were ampicillin (100 μg/ml) and tobramycin (5 μg/ml).

Preparation and analysis of DNA. Isolation of total DNA and small- and large-scale preparation of plasmid DNA was done as described previously (38). Electrophoresis was performed in 0.8% agarose gels (Sigma Chemical Co., St. Louis, Mo.) with a Tris-borate buffer system.

For the analysis of total DNA by pulsed-field gel electrophoresis (PFGE), strains were grown in 2-ml volumes of brain heart infusion broth. After centrifugation at 32 × *g* for 10 min, the cell pellet was suspended in 750 μl of 10 mM Tris base, 10 mM EDTA, 10 mM EGTA, 1 M NaCl (pH 7.5). Agarose plugs were

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristic(s)[a]	Source or reference
E. coli JM83	*ara*Δ(*lac* mutant *proAB*) *rpsL* [Φ80Δ(*lacZ*)*M15*]	48
S. maltophilia		
BM2690	Ak Gm Is Km Nt Tm	Clinical isolate
ATCC 13637	Ak Gm Is Km Nt Tm	Type strain
Plasmids		
pUC18	Tra$^-$ Mob$^-$ Ap	48
pAT680	Tra$^-$ Mob$^-$ Ak Is Km Nt Tm	1.1-kb *Pst*I fragment from BM2690 into pUC18
pAT681	Tra$^-$ Mob$^-$ Ak Is Km Nt Tm	903-bp *Eco*RI-*Pst*I fragment from pAT680 into pUC18

[a] Tra$^-$, non-self-transferable; Mob$^-$, non mobilizable; Ak, amikacin resistance; Ap, ampicillin resistance; Is, isepamicin resistance; Gm, gentamicin resistance; Km, kanamycin resistance; Nt, netilmicin resistance; Tm, tobramycin resistance.

made from a 1:1 mixture of 1.8% insert agarose (FMC BioProducts, Rockland, Maine) and the cell suspension. Each plug was placed in 1 ml of lysis buffer (6 mM Tris-HCl, 1 M NaCl, 100 mM EDTA, 0.5% Brij 58 [Sigma], 0.2% deoxycholic acid [Sigma], 0.5% *N*-lauroyl sarcosine [Sigma], 1 mg of lysozyme [Sigma]/ml) for 1 h at 37°C. Genomic DNA in agarose plugs was then treated for 16 h at 55°C with 1 ml of TE buffer (10 mM Tris base, 1 mM EDTA) containing 100 μg of proteinase K (Sigma). After three 1-h washes with TE buffer, the plugs were stored in TE buffer at 4°C. The plugs were digested with 20 U of restriction enzyme *Dra*I, *Xba*I, or *Spe*I (Boehringer, Mannheim, Germany) for 20 h at 37°C. They were then loaded into the wells of a 0.8% agarose gel in 45 mM Tris-borate, 1 mM EDTA (pH 8.0), and genomic DNA was separated by PFGE at 4.5 V/cm for 21 h at 14°C with a Chef DRIII apparatus (Bio-Rad, Ivry-sur-Seine, France). The pulse time was 5 to 50 s with linear ramping. Lambda ladder PFGE I (Boehringer) was incorporated as a size standard. The electrophoresis products were visualized by ethidium bromide staining for 30 min.

DNA techniques. For dot blotting and Southern hybridization, DNA was immobilized on Nytran membranes (Schleicher & Schuell, Dassel, Germany). Prehybridization and hybridization were carried out as described previously (38). The *aac(6′)-Iz*-specific probe was obtained by amplification of a 367-bp fragment by PCR from plasmid DNA obtained from *E. coli* JM83/pAT680 with primers 5′ TGTACCCGTGATCGCCA and 5′ ACTGTCCGAAGCCAGTT deduced from the sequence shown in Fig. 1. PCR was performed in a DNA Thermal Cycler 480 (Perkin-Elmer Cetus, Norwalk, Conn.) with primer annealing at 55°C, as described previously (28). The 367-bp fragment was separated by electrophoresis in low-temperature-gelling agarose type VII (Sigma), extracted and purified with a Qiagen (Chatsworth, Calif.) kit, and radiolabeled with [α-^{32}P]dCTP with the nick translation kit from Bethesda Research Laboratories Inc. (Gaithersburg, Md.) as described previously (38). The cDNA of the 16S and 23S rRNA of *E. coli* MRE 600 (Boehringer) was obtained with avian myeloblastosis virus reverse transcriptase (Boehringer) and radiolabeled with [α-^{32}P]dCTP according to the recommendations of the manufacturer. The physical link between *aac(6′)-Iz* and the *pdxH*-like gene was studied with primers C (5′ AGCCAGATTGGCGCGTGG) and D (5′ TAGACGACCCGTTCGGTCTC) located in these genes, respectively (Fig. 1), under PCR conditions similar to those indicated above.

DNA sequencing. The 1.1-kb *Pst*I fragment was cloned into bacteriophage M13 derivatives (Boehringer) and sequenced by the dideoxynucleotide chain terminator technique with synthetic oligonucleotides and the Sequenase version 2.0 DNA-sequencing kit (United States Biochemical Corp., Cleveland, Ohio) according to the recommendations of the manufacturer. DNA fragments were resolved by electrophoresis on 8% polyacrylamide gels containing 8 M urea. Nucleotide and amino acid sequences were analyzed and compared by using the GenBank, EMBL, and Swiss-Prot databases with the FASTA program (Genetics Computer Group, Madison, Wis.).

Analysis of plasmid-encoded proteins. The proteins specified by the recombinant plasmids were synthesized in a coupled in vitro transcription-translation system (50). The proteins were labeled with L-[^{35}S]methionine and processed for electrophoresis in a sodium dodecyl sulfate-polyacrylamide gel as described previously (38).

Enzymes and chemicals. T4 DNA ligase was from Amersham (Little Chalfont, Buckinghamshire, England), lysozyme was from Sigma Chemical Co., and RNaseA (bovine pancreas) was from Calbiochem-Behring (La Jolla, Calif.). [α-^{32}P]dCTP, [1-^{14}C]acetyl coenzyme A, and [α-^{35}S]dATP (400 Ci/mmol) were obtained from the Radiochemical Centre, Amersham. Antibiotics were provided by the following laboratories: amikacin, ampicillin, and kanamycin B, Bristol-Myers Squibb (Princeton, N.J.); tobramycin, Eli Lilly & Co. (Indianapolis, Ind.); gentamicin, gentamicins C1a, C1, and C2, isepamicin, netilmicin, 2′-*N*-ethylnetil-

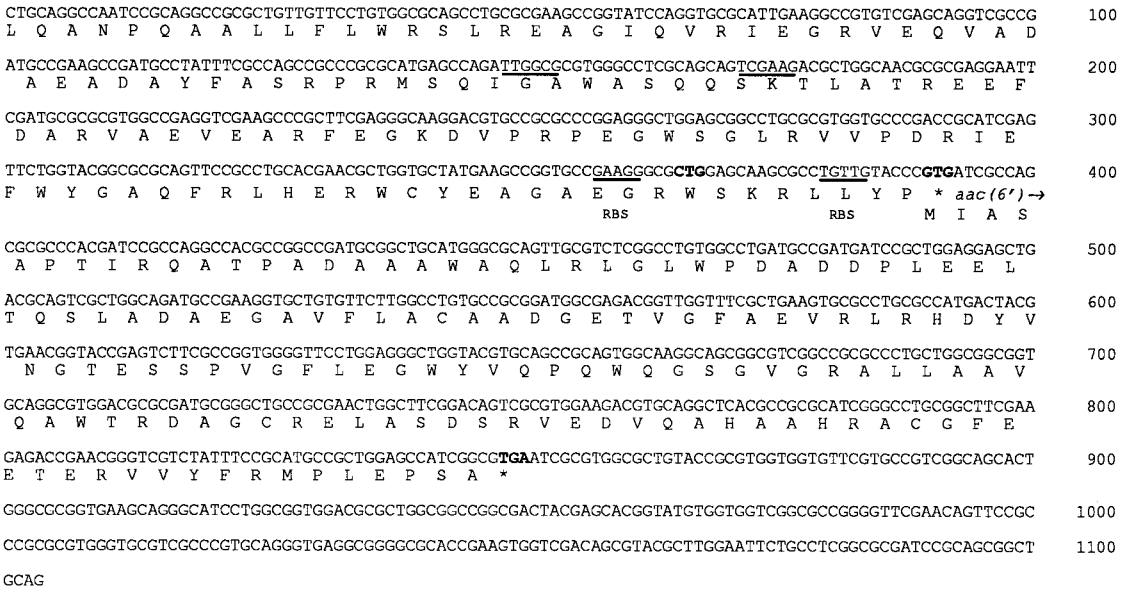

FIG. 1. Sequence of *S. maltophilia aac(6′)-Iz* and part of *pdxH*-like gene and predicted amino acid sequences. The putative ribosome binding site (RBS) and potential −35 and −10 promoter sequences are underlined. The start and stop codons are depicted in boldface, with the stop codons indicated by asterisks.

TABLE 2. MICs of various aminoglycosides against *S. maltophilia* BM2690 and *E. coli* JM83 with and without the *aac(6′)-Iz* gene

Strain	MIC (μg/ml)[a]				
	Amikacin	Gentamicin	Isepamicin	Netilmicin	Tobramycin
S. maltophilia BM2690	256	16	64	64	64
E. coli JM83	0.5	0.5	0.5	0.25	0.5
E. coli JM83/pAT680	4	1	2	4	16

[a] MICs determined on MH agar at 37°C.

micin, and 6′-*N*-ethylnetilmicin, Schering-Plough Research Institute (Kenilworth, N.J.); and nalidixic acid, Sterling Winthrop (New York, N.Y.).

Nucleotide sequence accession number. The nucleotide sequence of *aac(6′)-Iz* has been deposited in the GenBank data library under accession no. AF140221.

RESULTS AND DISCUSSION

Aminoglycoside resistance of *S. maltophilia* BM2690. The MICs of aminoglycosides against *S. maltophilia* BM2690 indicated that amikacin, isepamicin, netilmicin, and tobramycin were slightly less active against this strain than gentamicin (Table 2). Similar results were observed for most *S. maltophilia* strains (Table 3). The variability in aminoglycoside MICs could be due to alterations in penetration of the antibiotics in the cells, as has been observed in *Pseudomonas* spp. Disk-agar diffusion tests indicated that the activity of 2′-*N*-ethylnetilmicin against *S. maltophilia* BM2690 was diminished compared with that of 6′-*N*-ethylnetilmicin. These two compounds have similar intrinsic activities against aminoglycoside-susceptible strains, and the difference observed can be taken as evidence for production of a 6′-*N*-acetyltransferase.

Aminoglycoside-modifying enzyme in *S. maltophilia* BM2690. Extracts of *S. maltophilia* BM2690 were shown to contain aminoglycoside acetyltransferase activity. The substrate profile of the enzyme was consistent with an AAC(6′)-I, since amikacin, gentamicins C1a and C2, isepamicin, kanamycin B, netilmicin, tobramycin, and 2′-*N*-ethylnetilmicin were modified whereas gentamicin C1 and 6′-*N*-ethylnetilmicin were not (data not shown).

Cloning and sequencing of the *aac(6′)-I* gene. Total DNA from BM2690 and pUC18 DNA were digested with *Pst*I, mixed, ligated, and introduced by transformation into *E. coli* JM83. Transformants selected on medium containing ampicillin plus tobramycin were screened for their plasmid content by agarose gel electrophoresis of crude bacterial lysates. The


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AAC(6')-Ic         MIVICDHDNLDAWLALRTALWPSGSPEDHRAEMREILAS--PHHTA-FMARGLDGAFVAFAEVALRYDYVNGCES     72
AAC(6')-Id         MIEACHSVECPGWLQLRFLLWPQDSADEHLAEMAIFVAE--PNRFAQFIAYDEANKPLGFVEAALRSDYVNGTNS     73
AAC(6')-If             MDEASLSMWVGLRSQLWPDHSYEDHILDSQHILSC--PDKYVSFLAINNQSQAIAFADAAVRHDYVNGCES     69
AAC(6')-Ig        MNIKPASEASLKDWLELRNKLWSDS-EASHLQEMHQLLAE---KYALQLLAYSD-HQAIAMLEASIRFEYVNGTET     71
AAC(6')-Ih        MNIMPISESQLSDWLALRCLLWPDH-EDVHLQEMRQLITQ---AHRLQLLAYTDTQQAIAMLEASIRYEYVNGTQT     72
AAC(6')-Ij        MNIMPVSESLMADWLGLRKLLWPDH-DEAHLQEMQRLLQQ---TQSLQLLAYSDTQQAIAMLEASIRYEYVNGTQT     72
AAC(6')-Ik        MNIKPASEASLKDWLKLRIKLWNDL-EESHLQEMHQLLAE---KHALQLLVYSD-DHAVGMLEASIRYEYVNGTET     71
AAC(6')-Il    MDSSPLVRPVETTDSASWLSMRCELWPDGTCQEHQSEIAEFLSGKVARPAAVLIAVAPDGEALGFAELSIR-PYAEECYS     79
AAC(6')-Ir        MKIMPVSEPFLADWLQLRILLWPDHEEDAHLLEMRQLLEQ---PHTLQLLSYNDQQQAVAMLEASIRYEYVNGTQS     73
AAC(6')-Is        MNIMPISESQLSDWLALRSLLWPDH-EDAHLLEMRHVLKQ---TDTLQLLVYSETQLAIAMLEASIRHEYVNGTQT     72
AAC(6')-It        MHIMPITESQLSDWLVLRCLLWPDI-EDADLQEMRQLITQ---AHCLQLLAYTNTQKAIGMLEASIRYEYVNGTQT     72
AAC(6')-Iu        MNILPISESQLSDWLALRSLLWPDH-EEAHLQEMRQLLKQ---TDTLQLLAYSETQHAIAMLEASIRHEYVNGTQT     72
AAC(6')-Iv        MKIMPISESQLSDWLVLRCLLWPDH-EEQHLQEMRQLITQ---AHCLQLLAYTDTQQAIAMLEASIRYEYVNGTQT     72
AAC(6')-Iw        MKIMPISEALLADWLQLRILLWPDH-EDAHLLEMRQLLTR---TDSLQLLAYSETQQPIAMLEASIRHEYVNGTQT     72
AAC(6')-Ix        MNIMPISESQLSDWLALRSLLWPDH-DDAHLLEMHQLLKQ---TDTLQLLAYTDSQQAVAMLEASIRHEYVNGTQT     72
AAC(6')-Iz   MIASAPTIRQATPADAAAWAQLRLGLWPDA-DDP-LLELTQSLAD---AEGAVFLACAADGETVGFAEVRLRHDYVNGTES     76
                    •          *  •*  **•      •  •    •           •        ••  •  •*  * •


AAC(6')-Ic   SPVAFLEGIYTAERARRQGWAARLIAQVQEWAKQQGCSELASDTDIANLDSQRLHAALGFAETERVVFYRKTLG           146
AAC(6')-Id   SPVAFLEGVYVLPEARRRGIAHALVGAVEIWARNRACTEFASDASTDNPESHRFHQSLGFKETERVVYFRKMLAPE       149
AAC(6')-If   SPVVYLEGIFVIPEQRGHGVAKLLVAAVQDWGVAKGCTEMASDAALDNHISYQMHQALGFEETERVVFFRKRIAG        144
AAC(6')-Ig   SPVGFLEGIYVLPAHRRSGVATMLIRQAEVWAKQFSCTEFASDAALDNVISHAMHRSLGFQETEKVVYFSKKID         145
AAC(6')-Ih   SPVAFLEGIFVLPEYRRSGIATGLVQQVEIWAKQFACTEFASDAALDNQISHAMHQALGFHETERVVYFKKNIG         146
AAC(6')-Ij   SPVAFLEGIYVLPDYRRSGIATHLVQQVEAWAKPFGCIEFASDAALDNRISHAMHQALGFHETERVVYFKKHIG         146
AAC(6')-Ik   SPVAFLEGIYVLPEYRRLGVATLLVRQVEAWAKQFSCTEFASDAALDNVISHAMHRALGFQETERVVYFSKKID         145
AAC(6')-Il   GNVAFLEGWYVVPSARRQGVGVALVKAAEHWARGRGCTEFASDTQLTNSASTSAHLAAGFTEVAQVRCFRKPL          152
AAC(6')-Ir   SPVAFLEGIYVLPEYRRLGVASTLVQQVEHWAKQFACTEFASDAALDNTISHAMHRALGFQETECVVYFKKNIS         147
AAC(6')-Is   SPVAFLEGIYVLPEYRRSGFATQLVQCVEEWAKQFACTEFASDAALENTISHAMHRALGFHETERVVYFKKNIG         146
AAC(6')-It   SPVAFLEGIYVLPEYRRSGIATGLVQHVEIWAKQFACTEFASDATLDNQISHAMHQALGFHETERVVYFKKNIG         146
AAC(6')-Iu   SPVAFLEGIYVLPEYRRSGIATQLVQCVEEWAKQFACTEFASDAALDNTISHAMHRALGFHETERVVYFKKNIS         146
AAC(6')-Iv   SPVAFLEGIYVLPEYRRSGIATGLVQHVEIWAKQFSCTEFASDAALDNQITHAMHQALGFQETERVVYFKKNIG         146
AAC(6')-Iw   SPVAFLEGIYVLPEHRRSGIATQLVQQVEQWAKQYACTEFASDAAIDNTISHAMHQALGFHETERVVYFKKNIS         146
AAC(6')-Ix   SPVAFLEGIYILPEYRRSGIATQLVQYVEEWAKQFACTEFASDAAIDNTISHAMHRALGFHETERVVYFKKNIG         146
AAC(6')-Iz   SPVGFLEGWYVQPQWQGSGVGRALLAAVQAWTRDAGCRELASDSRVEDVQAHAAHRACGFEETERVVYFRMPLEPSA      153
             • * •***•         *•   *•   •  *     * * ***    •  •   * • ** *   *  •
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FIG. 2. Alignment of the deduced amino acid sequences of AAC(6′)-I. In addition to *S. maltophilia* AAC(6′)-Iz, the sequences are from *S. marcescens* [AAC(6′)-Ic] (41), *K. pneumoniae* [AAC(6′)-Id] (39), *E. cloacae* [AAC(6′)-If] (43), *Acinetobacter haemolyticus* [AAC(6′)-Ig] (22), *A. baumannii* [AAC(6′)-Ih] (23), *Acinetobacter* sp. 13 [AAC(6′)-Ij] (23), *Acinetobacter* sp. 6 [AAC(6′)-Ik] (34), the pBWH301 plasmid of *E. aerogenes* [AAC(6′)-Il] (4), *Acinetobacter* sp. 14 [AAC(6′)-Ir] (35), *Acinetobacter* sp. 15 [AAC(6′)-Is] (35), *Acinetobacter* sp. 16 [AAC(6′)-It] (35), *Acinetobacter* sp. 17 [AAC(6′)-Iu] (35), *Acinetobacter* ungrouped 631 [AAC(6′)-Iv] (35), *Acinetobacter* ungrouped 640 [AAC(6′)-Iw] (35), and *Acinetobacter* ungrouped BM2722 [AAC(6′)-Ix] (35). The dashes indicate gaps introduced to optimize sequence similarity. The alignments were derived with the Clustal V program (19). Identical amino acids are indicated by asterisks. The dots indicate conservative amino acid substitutions corresponding to the following exchange groups: A, G, P, S, and T; H, K, and R; F, W, and Y; D, E, N, and Q; I, L, M, and V; and C (11).

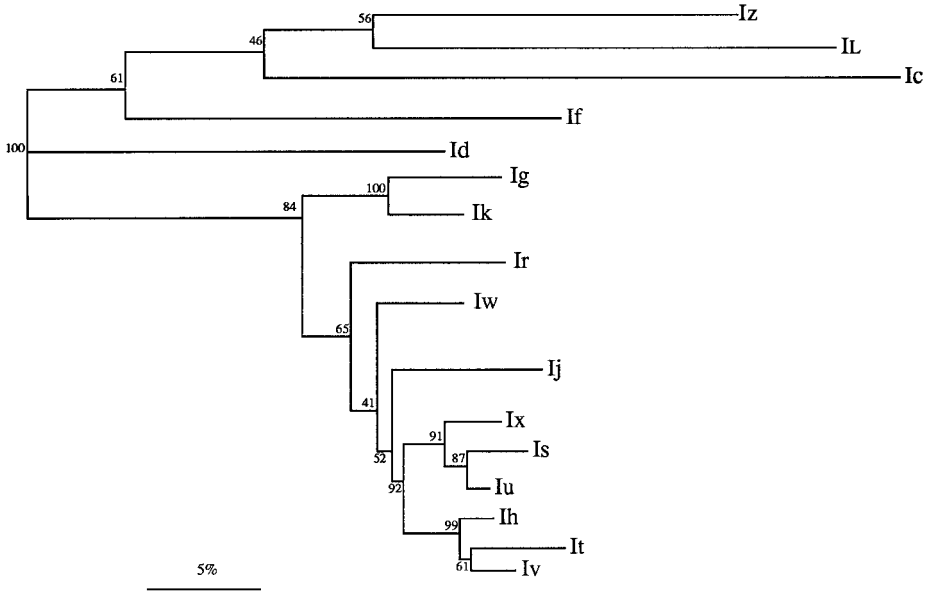

FIG. 3. Phylogenetic tree of the major subfamily AAC(6′)-I constructed with the Phylip computer program package (15) by the neighbor-joining method (37). The topology of the tree was compared to that obtained by the maximum-parsimony method (16). The numbers shown next to the nodes indicate percent bootstrap values of the 100 replicates (14). The line below the alignment indicates the distances that corresponds to approximately 5% divergence. In addition to *S. maltophilia* AAC(6′)-Iz, proteins are from *S. marcescens* [AAC(6′)-Ic] (41), *K. pneumoniae* [AAC(6′)-Id] (39), *E. cloacae* [AAC(6′)-If] (43), *Acinetobacter haemolyticus* [AAC(6′)-Ig] (22), *A. baumannii* [AAC(6′)-Ih] (23), *Acinetobacter* sp. 13 [AAC(6′)-Ij] (23), *Acinetobacter* sp. 6 [AAC(6′)-Ik] (34), the pBWH301 plasmid of *E. aerogenes* [AAC(6′)-Il] (4), *Acinetobacter* sp. 14 [AAC(6′)-Ir] (35), *Acinetobacter* sp. 15 [AAC(6′)-Is] (35), *Acinetobacter* sp. 16 [AAC(6′)-It] (35), *Acinetobacter* sp. 17 [AAC(6′)-Iu] (35), *Acinetobacter* ungrouped 631 [AAC(6′)-Iv] (35), *Acinetobacter* ungrouped 640 [AAC(6′)-Iw] (35), and *Acinetobacter* ungrouped BM2722 [AAC(6′)-Ix] (35).

smallest recombinant plasmid, pAT680, contained a 1.1-kb *Pst*I fragment that conferred aminoglycoside resistance by synthesis of an AAC(6′)-I. Analysis of the insert in pAT680 revealed two open reading frames (ORFs) homologous to sequences in the GenBank sequence library. Nucleotides 1 to 393 were 69% identical to the 3′ terminus of the *fprA* gene, which encodes a putative pyridoxaminephosphate oxidase in *Myxococcus xanthus* (18). Nucleotides 411 to 849 were 59% identical to the *aac(6′)-Ic* gene of *S. marcescens*. The search for stop codons indicated that the *aac(6′)-Iz* gene was included in an ORF located between the TGA codons at coordinates 335 and 849 (Fig. 1). Two potential translation initiation codons, CTG and GTG, were located at positions 366 and 390, respectively, but neither was preceded by a typical ribosome binding site. This may account for the low-level aminoglycoside resistance observed in *E. coli* JM83/pAT680 (Table 1). Promoter sequences were not readily apparent by analysis of the region upstream from *aac(6′)-Iz*; a putative promoter may consist of −35 (TTGGCG) (position 149 to 154) and −10 (TCGAAG) motifs separated by 17 nucleotides (the underlined nucleotides indicate identity with the consensus −35 and −10 promoter elements recognized by the *E. coli* σ^{70} factor). It is also possible that *aac(6′)-Iz* and a *pdxH*-like gene form a single transcription unit, similar to *aacC3* and *cysC* in *Pseudomonas aeruginosa* (44).

Analysis of the AAC(6′)-Iz protein. To confirm that the ORF between nucleotides 335 and 849 encodes the AAC(6′)-Iz pro-

TABLE 3. Susceptibility of 80 *S. maltophilia* strains to aminoglycosides

Aminoglycoside	MIC (μg/ml)[a]		
	Range	50%	90%
Amikacin	4→256	128	>256
Gentamicin	1→256	16	128
Isepamicin	4→256	64	256
Netilmicin	1→256	64	256
Tobramycin	1→256	32	256

[a] MICs determined on MH agar at 37°C.

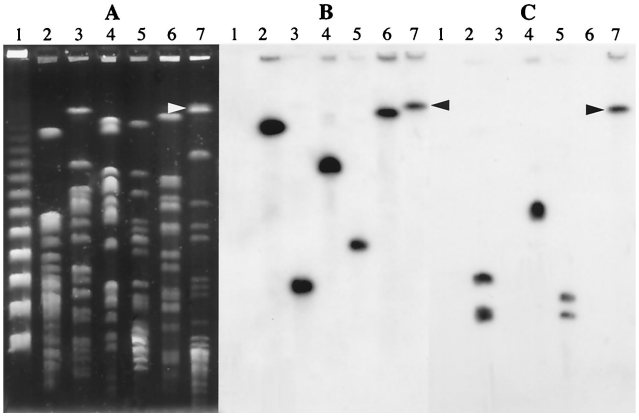

FIG. 4. Analysis of total DNA by PFGE (A) and by hybridization with probes made from *aac(6′)-Iz* (B) and rRNA (C). Total DNA was digested with *Xba*I, *Dra*I, or *Spe*I, and the resulting fragments were separated by PFGE, transferred to a Nytran filter, and hybridized with the ^{32}P-labeled probes. Lanes: 1, PFGE I ladder (Boehringer Mannheim); 2, 3, and 4, *S. maltophilia* BM2690 digested with *Xba*I, *Dra*I, and *Spe*I, respectively; 5, 6, and 7, *S. maltophilia* ATCC 13637 digested with *Xba*I, *Dra*I, and *Spe*I, respectively. The arrowheads indicate the *S. maltophilia* ATCC 13637 fragment containing the *aac(6′)-Iz* which hybridized with the rRNA probe. The lack of *Dra*I fragments hybridizing with the rRNA probe was due to their migration out of the gel.

tein, a 903-bp *Eco*RI-*Pst*I fragment was subcloned into pUC18, generating pAT681. The proteins specified by pUC18 and pAT681 were characterized in an *E. coli* in vitro coupled transcription-translation system. A band of approximately 16,000 Da, which should correspond to the resistance protein, was encoded by pAT681 but not by the pUC18 control (data not shown). This apparent molecular mass was in good agreement with the 16,506 Da calculated for the predicted amino acid sequence. On the basis of sequence relationships, three subfamilies of AAC(6′) have been distinguished. AAC(6′)-Iz is a member of the major subfamily which contains AAC(6′)-Ic specific for *S. marcescens* (41) (46% identity); AAC(6′)-Id found in *Klebsiella pneumoniae* (39) (42% identity); AAC(6′)-If from *Enterobacter cloacae* (43) (45% identity); AAC(6′)-Il from *Enterobacter aerogenes* (4) (44% identity); AAC(6′)-Ih from *Acinetobacter baumannii* (23) (42% identity); and AAC(6′)-Ig, -Ij, -Ik, -Ir, -Is, -It, -Iu, -Iv, -Iw, and -Ix specific for the various species of proteolytic *Acinetobacter* (22, 23, 34, 35) (42 to 46% identity) (Fig. 2). The relatedness of the major subfamily AAC(6′)-I is shown in Fig. 3.

Analysis of the protein deduced from the second ORF. The deduced C-terminal portion of the putative protein encoded by the truncated DNA sequence upstream from the *aac(6′)-Iz* gene had homology with pyridoxine (pyridoxamine) 5′-phosphate oxidase proteins found in *M. xanthus* (18) (61% identity), *Saccharomyces cerevisiae* (24) (43% identity), and *E. coli* (21) (39% identity). It is likely that this DNA corresponds to the analogous gene in *S. maltophilia*. Pyridoxine (pyridoxamine) 5′-phosphate oxidase is a key enzyme in biosynthesis of the essential coenzyme pyridoxal 5′-phosphate, which participates in many aspects of amino acid metabolism. The fact that, in *S. maltophilia* BM2690, *aac(6′)-Iz* was adjacent to a gene involved in essential host metabolism strongly suggests a chromosomal location for that gene. Furthermore, the G+C content of the *aac(6′)-Iz* was 69% similar to that of *S. maltophilia*, in agreement with an indigenous origin of this gene.

Distribution and location of the *aac(6′)-Iz* gene. Total DNA from 80 *S. maltophilia* strains was spotted on a Nytran membrane and hybridized with an intragenic *aac(6′)-Iz* probe. The gene was found to be present in all the strains (data not shown). To determine the location of the *aac(6′)-Iz* gene, PFGE was performed with total DNA of *S. maltophilia* BM2690 and ATCC 13637 digested with *Xba*I, *Dra*I, and *Spe*I. The fragments were transferred to Nytran membranes and hybridized successively with an rRNA and an *aac(6′)-Iz* probe (Fig. 4). The *aac(6′)-Iz* gene was located in a ca. 510-kb *Xba*I fragment in *S. maltophilia* BM2690 and in a ca. 680-kb *Spe*I fragment in *S. maltophilia* ATCC 13637 which cohybridized with the rRNA probe. These results confirmed that the *aac(6′)-Iz* gene was located in the chromosome of *S. maltophilia*. In addition, the adjacent location of *aac(6′)-Iz* and *pdxH*-like genes in the remaining strains of *S. maltophilia* was demonstrated by amplification of a 676-bp fragment with primers C, specific for *pdxH*, and D, specific for *aac(6′)-Iz* (data not shown).

Conclusions. Like *S. marcescens* (41), *E. faecium* (6), and the various species of proteolytic *Acinetobacter* (35), *S. maltophilia* harbors a "housekeeping" *aac(6′)-I*. This gene, probably weakly expressed, contributes to aminoglycoside resistance in this species as indicated by the slight difference in susceptibility to gentamicin and to the other aminoglycosides (Table 3). The origin of aminoglycoside resistance genes is still a matter of controversy (33). A possibility is that they derive from antibiotic-producing microorganisms in which they are required for self-protection against the toxic aminoglycosides (3, 7, 10). Alternatively, they could derive from housekeeping genes involved either in cellular metabolism or in peptidoglycan or lipopolysaccharide metabolism (27). These two hypotheses are not mutually exclusive, and there are strong arguments in favor of each proposal, depending on the bacterial species considered. It has been established that the resident AAC(2′)-Ia in *Providencia stuartii* can O-acetylate peptidoglycan (27, 32), and a similar function for the AAC(2′)-Id of *Mycobacterium segmatis* is possible (1). The functional role, if any, of AAC(6′)-Iz in *S. maltophilia* remains unknown.

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