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Cloning and Sequencing of a Gene Encoding an Aminoglycoside 6′-*N*-Acetyltransferase from an R Factor of *Citrobacter diversus*

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The *aacA1* gene, which encodes a 6′-*N*-acetyltransferase [AAC(6′)-I] mediating resistance to kanamycin, tobramycin, and amikacin, was cloned from the *Citrobacter diversus* R plasmid pBWH100 into the *Escherichia coli* vector pBR322. The complete nucleotide sequence of the gene and flanking regions was determined. A protein of approximately 21 kilodaltons was identified when the chimeric plasmid encoding the *aacA1* gene was introduced into *E. coli* maxicells. This value is consistent with the size predicted for a protein translated from the open reading frame of the gene.

The most common mechanism of resistance to the aminoglycoside-aminocyclitol antimicrobial agents among bacteria is enzymatic modification of the antibiotic (7, 8, 22, 23, 28). This can occur by adenylylation, phosphorylation, or acetylation. The aminoglycoside acetylases, including the AAC(6′), AAC(3), and AAC(2′), are a diverse group of enzymes (8, 22, 23). Only two genes related to these enzymes, both encoding AAC(3)-type enzymes, have been sequenced (1, 3). The AAC(6′)-I enzyme, which mediates resistance to amikacin, kanamycin, and tobramycin (7, 8, 22), has been noted in *Serratia marcescens* (15, 17, 18, 22, 28), *Escherichia coli* (10, 26), *Citrobacter diversus*, *Klebsiella pneumoniae*, *Enterobacter cloacae* (8, 20–22, 28), and *Pseudomonas aeruginosa* (9, 13, 16, 18, 28).

To initiate studies of the epidemiology of the 6′-*N*-acetyltransferase gene, we cloned and sequenced the *aacA1* gene from the R factor pBWH100 (*E. coli* PS2225), a 92-kilobase R factor that also carries a TEM-1 type β-lactamase gene and a sulfonamide resistance gene (20). This R factor, originally isolated from a strain of *C. diversus* obtained from sputum from a patient at the Brigham and Women's Hospital in Boston, Mass., was provided by K. Mayer, Providence, R.I. All chimeric plasmids were transformed into either *E. coli* C600 (19) or *E. coli* JC2926 (19) by the method of Cohen et al. (5). The presence of the AAC(6′)-I gene in the original strain containing pBWH100 and in several subsequent *E. coli* strains harboring subclones of the gene was confirmed by the MIC typing method of Miller et al. (22), performed by R. Hare of Schering Corp., Bloomfield, N.J.

All restriction endonucleases and T4 DNA ligase were purchased from New England BioLabs, Beverly, Mass., and were used according to the directions of the manufacturer.

Plasmid-encoded proteins were examined in *E. coli* JC2926 maxicells by using the procedure of Sancar et al. (27). Proteins were labeled with [^{35}S]methionine (1,112 Ci/mmol; New England Nuclear Corp., Boston, Mass.) and analyzed on 12% sodium dodecyl sulfate-polyacrylamide gels as previously described (29).

Plasmid pFCT1163 was digested with *Hin*dIII and *Pvu*II or *Hin*dIII and *Rsa*I, and the fragments were cloned into M13mp10 or M13mp11 (25). The procedure of Dale et al. (6) was used to progressively shorten some of the large inserts. The bacteriophage clones were sequenced by using the dideoxy method of Biggin et al. (2) with the substitution of deoxy-7-deazaguanosine triphosphate (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) for dGTP (24). The DNA sequence was determined independently on both strands throughout.

The *aacA1* gene was isolated from the plasmid pBWH100 and inserted into the *Hin*dIII site of the vector pBR322 (Fig. 1). This chimeric plasmid pFCT1063, when transformed into *E. coli*, conferred the AAC(6′)-I phenotype of resistance to amikacin, tobramycin, and kanamycin to the host strain. A *Hin*dIII-*Pvu*II subclone of pFCT1063, designated pFCT1163, also conferred the resistance phenotype to its host. The DNA sequence of the *Hin*dIII-*Pvu*II fragment is shown in Fig. 2. To confirm the assignment of promoter sequences at bases 577 through 605 (TTGTTA-17 bases-TACAGT) (11), a chimeric plasmid was constructed in which the 94-base-pair *Ava*I-*Ava*II fragment containing

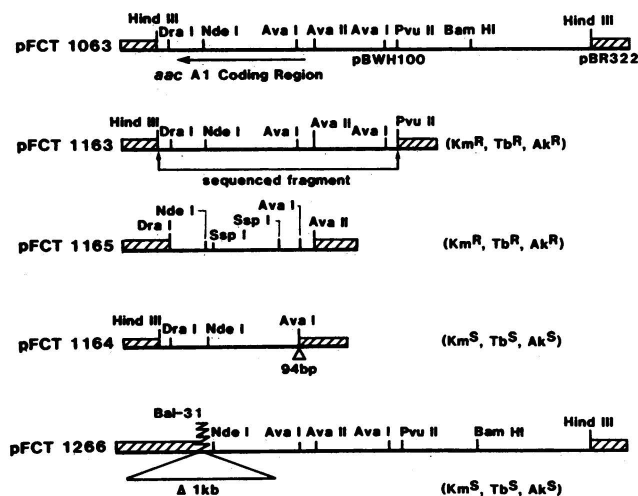

FIG. 1. Restriction endonuclease maps of chimeric plasmids constructed in this study. Symbols: ▨, regions of plasmid pBR322; ——, cloned DNA from pBWH100. Km^R, Plasmid produces kanamycin resistance phenotype in host strain of *E. coli*; Km^S, produces kanamycin susceptibility phenotype; Tb, tobramycin; Ak, amikacin; Δ, deletion.

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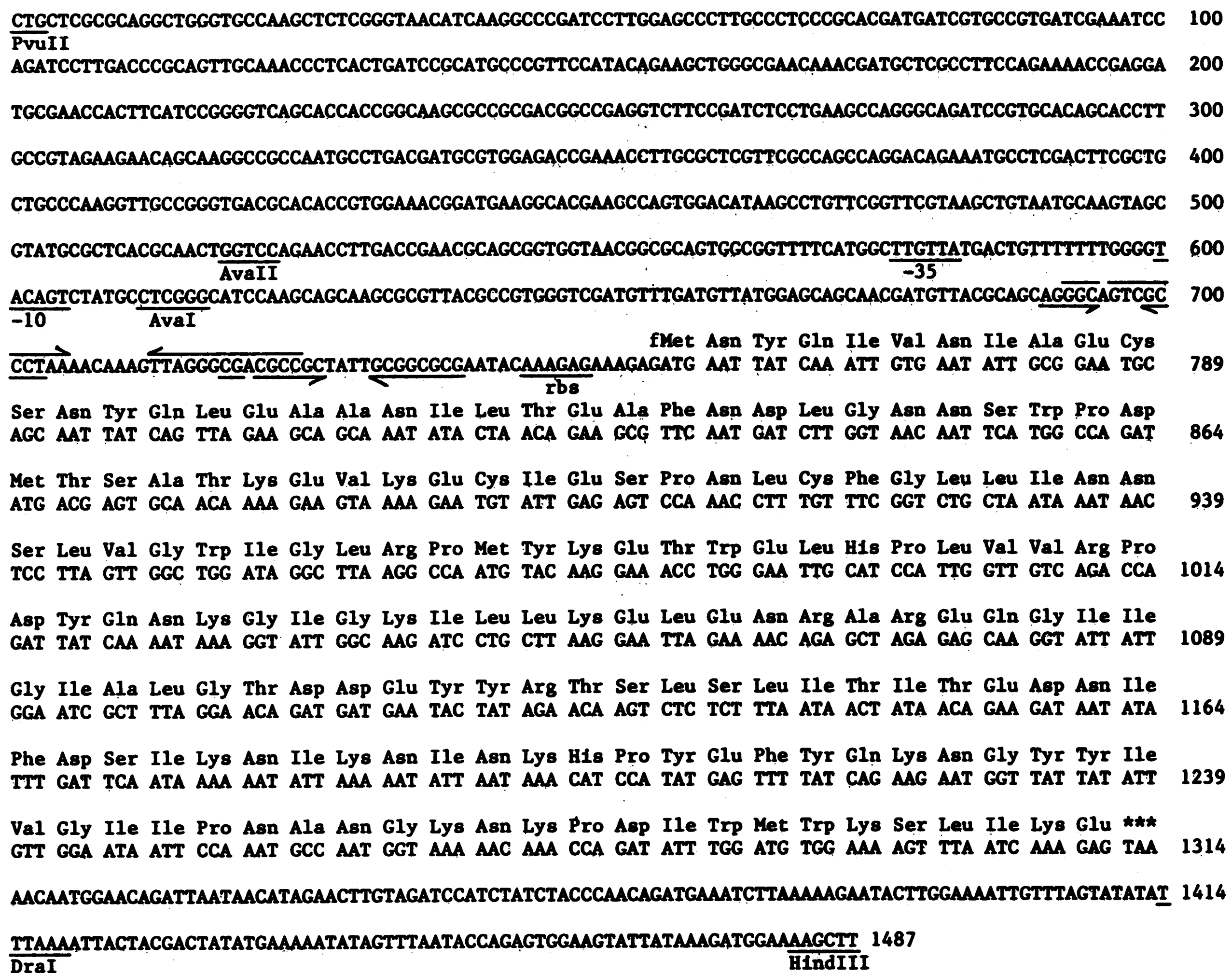

FIG. 2. DNA sequence of the cloned *Hin*dIII-*Pvu*II fragment of pBWH100 and its proposed translation. −10 and −35, proposed promoter sequences; rbs, proposed ribosome-binding sequence; ***, stop codon. Three regions of dyad symmetry are indicated by facing arrows.

these −35 and −10 sequences was deleted. This plasmid, pFCT1164, did not produce the AAC(6′)-I phenotype in host strains of *E. coli* when introduced into the cell by transformation. There are two potential initiation codons, the ATG at position 757 and the GTG at position 772. The 3′ end of the gene was determined by BAL 31 nuclease mapping studies to be the TAA located at position 1312.

The DNA sequence predicts a protein of 21,240 daltons with an amino acid sequence of 185 residues. To confirm the size of the protein produced by the cloned gene, chimeric plasmids containing the *aacA1* gene were introduced into an *E. coli* maxicell strain. A 20.5-kilodalton (kDa) protein was produced by *E. coli* containing the plasmids pFCT1163 and pFCT1165, but not by strains containing pFCT1164. The size of this protein is of particular interest since it is significantly smaller than the other AAC(6′) proteins previously reported (21). We have shown that the *aacA1* gene of pBWH100 is homologous to the 6′-*N*-acetyltransferase gene carried by *E. coli* W677 harboring the R5-NR79 R factor (10) under stringent conditions (data not shown). Meyer and Wiedemann (21) were unable to determine the size of the AAC(6′) protein produced by this strain, but Radika and Northrop (26) have reported that the same AAC(6′) gene produced a tetrameric protein of 60 to 70 kDa, composed of 14.5-kDa subunits. We found no evidence of proteins larger than 21 kDa during these studies.

Examination of the DNA sequence of the *aacA1* gene has revealed three interesting features. First, the translational terminator of the AAC(6′)-I protein at base pair 1312 overlaps another potential ribosome-binding site and open reading frame beginning at base pair 1306. This suggests that the *aacA1* gene may be part of an operon. Second, just upstream from the translational start of the *aacA1* gene at base pair 757 is a region of three inverted repeat structures (Fig. 2). These regions of dyad symmetry can form two mutually exclusive secondary structures and may serve as transcriptional attenuators (14). The role of this region of dyad symmetry is under investigation. Finally, the first 717 base pairs of the region upstream from the *aacA1* gene sequence are nearly identical (only three base pair differences) with the regions upstream from the *aadA* (12), *aadB* (4), and *dhfr*II (30) genes previously reported in the literature. The significance of this observation is unknown.

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