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Genetic Environment and Transcription of *ampC* in an *Acinetobacter baumannii* Clinical Isolate

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An *ampC* gene was cloned from a clinical isolate of *Acinetobacter baumannii* (strain RAN). DNA sequencing and primer extension studies showed that *ampC* is transcribed from a promoter contained within a putative insertion sequence element which has been found to abut several different genes in *Acinetobacter* spp.

Acinetobacter baumannii has emerged as a significant nosocomial pathogen, especially in intensive care units (4). The treatment of infections due to this organism can present therapeutic problems, primarily because *A. baumannii* is often resistant to multiple antibiotics, including the β-lactam antibiotics (11, 12). A number of chromosomal β-lactamases have been described in *A. baumannii* (2, 13); however, only the chromosomal cephalosporinase gene, *ampC*, has been cloned from this organism (5). As the transcription site of *ampC* was not established, the promoter for this gene is unknown (5). This communication describes the transcription and genetic environment of *ampC* from a clinical isolate of *A. baumannii* (strain RAN).

Strain RAN was isolated from blood and identified in the microbiology laboratory of Groote Schuur Hospital, Cape Town, South Africa. MICs of β-lactams with Etest strips (AB Biodisk, Solna, Sweden) for strain RAN are shown in Table 1. Similar determinations showed that strain RAN was resistant to amoxicillin/clavulanate (MIC, >256 μg/ml) and piperacillin/tazobactam (MIC, >256 μg/ml). The MIC of cephalothin (Sigma) was determined with a twofold agar dilution method on IsoSensitest agar (Oxoid, Basingstoke, United Kingdom) and an inoculum of 10^4 CFU/spot.

Genomic DNA from *A. baumannii* strain RAN was extracted (3), digested with *Hin*dIII, and ligated to similarly digested pBGS8 (17). Recombinant plasmids were introduced into *Escherichia coli* JM109 (18), which were made competent for DNA uptake with the $CaCl_2$ shock procedure (7). Recombinant plasmid DNA was extracted (15), and one plasmid, designated pMERL100, containing a *Hin*dIII insert of approximately 5.0 kb conferred resistance to cephalothin and cefuroxime and diminished susceptibility to cefotaxime and cefoxitin on its *E. coli* host (Table 1). This resistance phenotype is different from that of *E. coli* containing the *A. baumannii* *ampC* gene in a similar vector (pBGS18) designated pGER1 (5) (Table 1). The cefoxitin MIC (24 μg/ml) for *E. coli* (pMERL100) (Table 1) suggested activity against this antibiotic. However, with a spectrophotometric assay, hydrolysis of this antibiotic was not demonstrated for β-lactamase extracts of *E. coli*(pMERL100) (data not shown).

A portion (3.123 kb) of the 5.0-kb insert was sequenced on both strands (accession no. AY325306). The deduced amino acid sequence of the *ampC* structural gene showed 98.4, 98.7, 98.7, and 99% identity to *ampC* from *A. baumannii* RYC52763/97 (5), *A. baumannii* ABAC1, *A. baumannii* ABAC2 (14), and *Oligella urethralis* COH-1 (14), respectively. Sequencing data showed that the 5′ end of the structural *ampC* gene is adjacent to a sequence, called the homologous sequence, which contains features of insertion sequence elements, including outwardly directed promoters, found at the 5′ ends of a number of genes from *Acinetobacter* spp. (16). The *ampC* start codon is 9 bp downstream from the nucleotide (G) which defines the boundary of the homologous sequence (16).

The precise initiation site of the *ampC* transcript was determined by primer extension analysis. Total RNA was extracted from exponential-phase cultures of *A. baumannii* strain RAN, containing cefoxitin (30 μg/ml), with hot acidic phenol (9). A primer (5′-TACCTGGCACATCATATT-3′) that annealed to sequences downstream of the ATG initiation codon of the *ampC* gene was synthesized and labeled with indodicarbocyanine in the Department of Molecular and Cell Biology, University of Cape Town, Cape Town, South Africa. The primer was annealed to the mRNA template and extended with Moloney murine leukemia virus reverse transcriptase (Promega, Madison, Wis.) and an equimolar deoxynucleoside triphosphate mixture. The primer extension products were precipitated, reconstituted in ALF stop buffer (Pharmacia Biotech),

TABLE 1. β-Lactam MICs for *A. baumannii* strain RAN and comparison of MICs for *E. coli*(pMERL100) and *E. coli*(pGER1)[a]

Antibiotic	MIC (μg/ml)				
	A. baumannii RAN	*E. coli* JM109 (pMERL100)	*E. coli* JM109	*E. coli* TG1 (pGER1)	*E. coli* TG1
Cefuroxime	>256	32	2	>256	4
Cefoxitin	>256	24	3	4	4
Cefotaxime	>32	0.25	0.025	4	≤0.125
Ceftazidime	16	0.75	0.25	16	≤0.125
Cephalothin	>128	>128	ND[b]	ND	ND

[a] pMERL100 carries *ampC* cloned from *A. baumannii* RAN. pGER1 carries *ampC* cloned from *A. baumannii* RYC52763/97 (5).
[b] ND, not determined.

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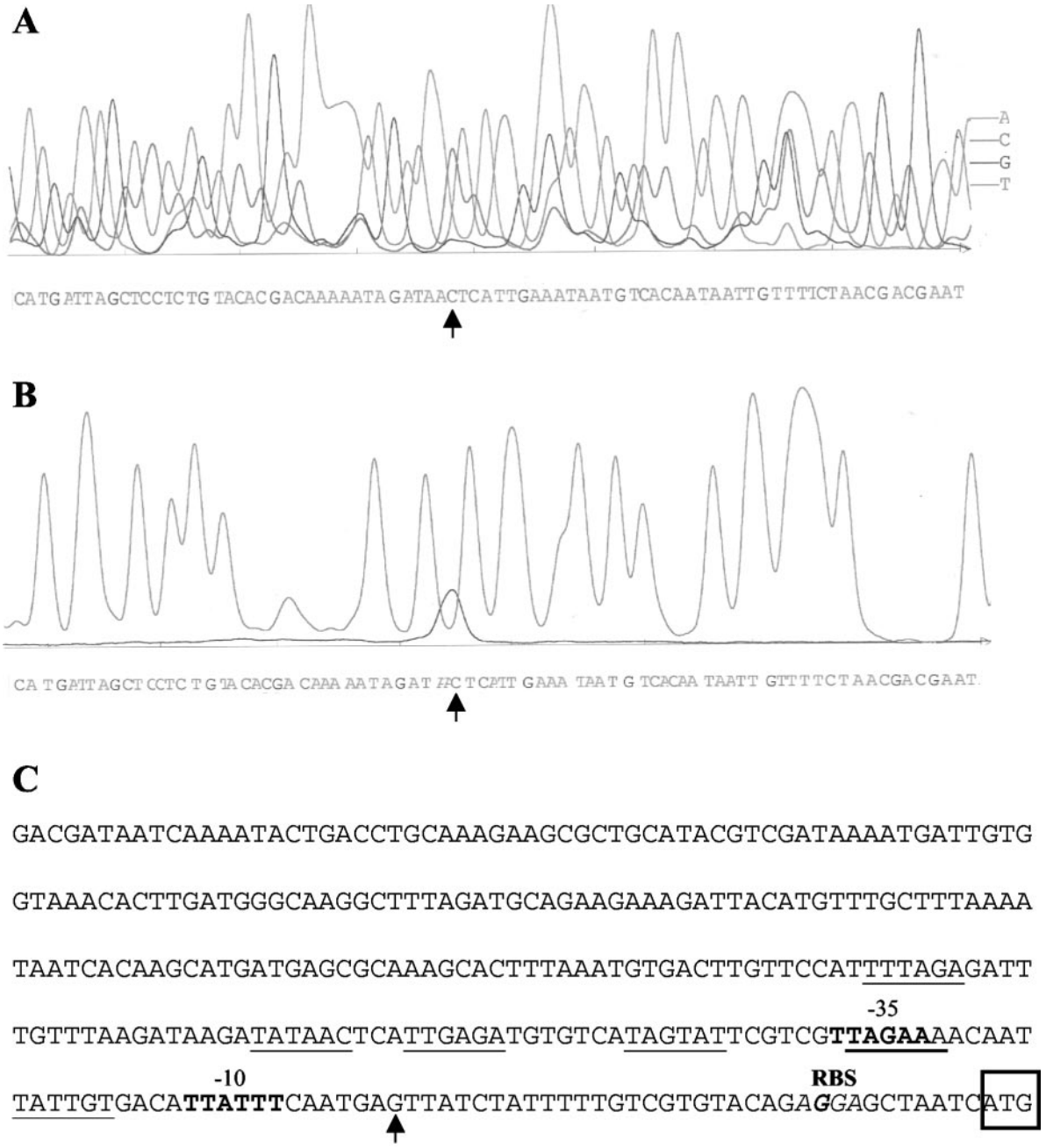

FIG. 1. Mapping of transcription initiation site of *A. baumannii* RAN *ampC* by primer extension analysis. DNA sequencing fluorograms generated with indodicarbocyanine-labeled primer are shown, with the peaks corresponding to the nucleotides indicated. (A) DNA sequence of template strand upstream of *ampC* initiation codon, starting at the initiation codon (CAT). (B) T sequencing reaction products and primer extension product. The transcription start site is indicated by the vertical arrow. (C) Nucleotide sequence of the regulatory region (300 bp) upstream of the *ampC* initiation codon (boxed). TTAGAA (−35)-N_{16}-TTATTT (−10) upstream of the transcription start site are shown in bold. Additional −35 and −10 hexamers are underlined. The coincident G demarcating the start of the homologous region is shown in bold. The ribosome-binding site (RBS) is indicated in italics.

heated, and analyzed in conjunction with sequencing reaction products performed on the corresponding DNA. Analysis of the products was performed with the ALFexpress automated DNA sequencer (Amersham Biosciences) in the Department of Molecular and Cell Biology, University of Cape Town.

The primer extension product was mapped to a G located in the putative insertion sequence, 35 nucleotides upstream of the *ampC* start codon (Fig. 1). The hexamers TTAGAA (−35) and TTATTT (−10), separated by 16 bp, upstream of the transcription start site show similarity to promoter consensus sequences recognized by $E\sigma^{70}$. By and large, there is good correlation between promoter strength and the degree of

similarity to the $E\sigma^{70}$ consensus hexamers TTGACA (−35) TATAAT (−10) and to the consensus length (17 bp) separating them (8). In terms of these parameters, the *ampC* promoter is weak. Different, properly aligned promoters (Fig. 1) are present in the putative insertion sequence upstream of *ampC*; thus, although only a single primer extension product was obtained, it is possible that transcription initiation could proceed from more than one promoter, resulting in an increase in AmpC production. Interestingly, hyperproduction of AmpC in *A. baumannii* strains has been reported recently (6). The hyperproducing strains were resistant to ceftazidime, and the *ampC* genes were located adjacent to the putative insertion sequence element (6), supporting the notion that transcription from different promoters within this element could result in an increase in AmpC.

The *ampC*-type gene $bla_{ABA\text{-}1}$ in *O. urethralis*, suggested to have originated in *A. baumannii*, was identified downstream of a different insertion sequence element, IS*Our1* (14). Putative promoters identified for the *ampC* in *O. urethralis* suggest that it is transcribed from a hybrid promoter, the −35 hexamer being located in the insertion sequence element. Subsequently, this sequence was identified in *A. baumannii* strains (6). Taken together, these data indicate that *ampC*-type genes are regulated differently in disparate *Acinetobacter* strains.

As different genes were identified downstream of the homologous region detected in *A. baumannii* strain PAU (16), we determined whether *ampC* is located in a different genetic environment in this strain. PCR assays with primers complementary to sequences in the homologous region (5′-ATAGT TTCACCCGACCA-3′) and *ampC* (P1) (5) showed that in strain PAU, *ampC* is adjacent to the homologous region (data not shown). Thus, this strain contains at least two copies of the homologous region or putative insertion sequence. A more intensive study is being carried out to determine the prevalence of the putative insertion sequence element, and the identity of the sequences flanking this region, in *A. baumannii* strains.

The DNA sequence (1,017 bp) at the 3′ end of *ampC* showed no similarity to the corresponding region of the previously described *ampC* (5). The sequence contains one open reading frame of 960 bp; the translation product of this open reading frame has 52% identity with the C terminus (amino acids 477 to 792) of ComA from *Acinetobacter calcoaceticus* BD413. In the naturally competent *A. calcoaceticus* BD413, ComA has been proposed to be involved with DNA transfer across the cytoplasmic membrane during the period of competence (10). The protein from strain RAN is much shorter (320 amino acids) than its counterpart (793 amino acids) in *A. calcoaceticus*, suggesting that it is one of the shorter homologues which has been described from a large number of bacterial species (http://tigrblast.tigr.org/web-hmm/accession no. TIGR00361). The function of the shorter homologues is unknown, and for the most part they have been identified in bacteria that are not known to be naturally competent, suggesting that strain RAN is not naturally competent for the uptake of DNA. Whether this applies to all *A. baumannii* strains remains to be determined.

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