ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2007, p. 3040–3041 Vol. 51, No. 8
0066-4804/07/$08.00+0 doi:10.1128/AAC.00594-07
Copyright © 2007, American Society for Microbiology. All Rights Reserved.

# Extended −10 Promoter in IS*Aba-1* Upstream of *bla*$_{OXA-23}$ from *Acinetobacter baumannii*$^{\triangledown}$

Molecular studies showed that carbapenem-resistant *Acinetobacter baumannii* strains isolated from patients in hospitals in Cape Town contain *bla*$_{OXA-23}$ (7). Concordant with the findings of others (4, 11), PCR assays showed that of the 43 strains investigated, *bla*$_{OXA-23}$ was consistently associated with IS*Aba-1*. One strain, designated RAM (8), was selected to identify the transcription promoter located upstream of *bla*$_{OXA-23}$.

Total RNA was extracted from strain RAM using the hot acid phenol method (1), and primer extension studies were carried out (9). The primer extension products were analyzed in conjunction with products of sequencing reactions performed on the corresponding DNA. The extension product mapped to a T 63 nucleotides upstream of the *bla*$_{OXA-23}$ start codon (Fig. 1). The hexamers TTAGAA (−35) and TTATTT (−10) are upstream of this start site. The sequence (TGACA) immediately upstream of the −10 box generates an extended −10 hexamer that shows similarity to sequences recognized by the $\sigma^S$ subunit of RNA polymerase (5, 6, 12), chief regulator of the general stress response in *Escherichia coli* (5, 12, 13). Further, a putative distal UP element half site (GTATTTGTTT) and a CAP site, thought to play a role in $\sigma^S$ selectivity (3, 12, 13), are upstream of the −10 box. It is noteworthy that RAM contains at least two other IS*Aba-1*-linked resistance genes (*sul2* and *ampC*). Primer extension studies indicated that the extended promoter also drives transcription of *sul2* (2). Albeit in a different strain of *A. baumannii*, this promoter was identified upstream of *ampC* (9) and it is assumed to be transcriptionally active with respect to *ampC* in strain RAM. The copy number and implied mobility of IS*Aba-1* in *Acinetobacter* spp. (8), combined with its repertoire of regulatory sequences, suggest that this element may play a significant role in controlling the expression of a variety of genes in *Acinetobacter*, even within a single strain of this organism.

As transcription signals used in *Acinetobacter* are different from their counterparts in *E. coli* (10), primer extension studies were carried out to study the expression of *bla*$_{OXA-23}$ in *E. coli*. The functional *bla*$_{OXA-23}$ gene was amplified from RAM; cloned into pGEM-T EASY, generating pRK001; and expressed in *E. coli* JM109. Using RNA from *E. coli*(pRK001), five transcription start sites were identified (Fig. 1), corresponding to two major and three minor peaks. One of the major peaks mapped to a T which equates to the transcription start site identified upstream of *bla*$_{OXA-23}$ in strain RAM; presumably, the transcript in *E. coli*(pRK001) also emanates from the extended −10 promoter. A different IS*Aba-1* promoter is present upstream of the second major start site (Fig. 1). These data indicate that although *A. baumannii* and *E. coli* possess related transcription machinery, at least one of the IS*Aba-1*-located promoters is more active in *E. coli* than in *A. baumannii*.

## REFERENCES


1. **Aiba, H., S. Adhya, and B. D. Crombugghe.** 1981. Evidence for two functional *gal* promoters in intact *Escherichia coli* cells. J. Biol. Chem. **25:**11905–11910.
2. **Garny, S.** 2006. Distribution, frequency and contribution to the expression of antibiotic resistance gene of an IS element in *Acinetobacter baumannii*. M.S. thesis, University of Cape Town, South Africa.
3. **Germer, J., G. Becker, M. Metzner, and R. Hengge-Aronis.** 2001. Role of activator site position and a distal UP-element half-site for sigma factor selectivity at a CRP/H-NS-activated $\sigma^S$-dependent promoter in *Escherichia coli*. Mol. Microbiol. **41:**705–716.
4. **Héritier, C., L. Poirel, T. Lambert, and P. Nordmann.** 2005. Contribution of acquired carbapenem-hydrolyzing oxacillinases to carbapenem resistance in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **49:**3198–3202.
5. **Lacour, S., and P. Landini.** 2004. $\sigma^S$-dependent gene expression at the onset of stationary phase in *Escherichia coli*: function of $\sigma^S$-dependent genes and identification of their promoter sequences. J. Bacteriol. **186:**7186–7195.
6. **Mitchell, J. E., D. Zheng, S. J. W. Busby, and S. D. Minchin.** 2003. Identification and analysis of 'extended −10' promoters in *Escherichia coli*. Nucleic Acids Res. **31:**4689–4695.
7. **Segal, H., and B. G. Elisha.** 2005. Use of Etest MBL strips for the detection of carbapenemases in *Acinetobacter baumannii*. J. Antimicrob. Chemother. **56:**598.
8. **Segal, H., S. Garny, and B. G. Elisha.** 2005. Is IS*ABA-1* customized for *Acinetobacter*? FEMS Microbiol. Lett. **243:**425–429.
9. **Segal, H., E. C. Nelson, and B. G. Elisha.** 2004. Genetic environment and transcription of *ampC* in an *Acinetobacter baumannii* clinical isolate. Antimicrob. Agents Chemother. **48:**612–614.
10. **Segal, H., and B. G. Elisha.** 1999. Characterization of the *Acinetobacter* plasmid, pRAY, and the identification of regulatory sequences upstream of an *aadB* gene cassette on this plasmid. Plasmid **42:**60–66.
11. **Turton, J. F., M. E. Ward, N. Woodford, M. E. Kaufmann, R. Pike, D. M. Livermore, and T. L. Pitt.** 2006. The role of IS*Aba1* in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. FEMS Microbiol. Lett. **258:**72–77.
12. **Typas, A., and R. Hengge.** 2005. Differential ability of $\sigma^S$ and $\sigma^{70}$ of *Esche-*


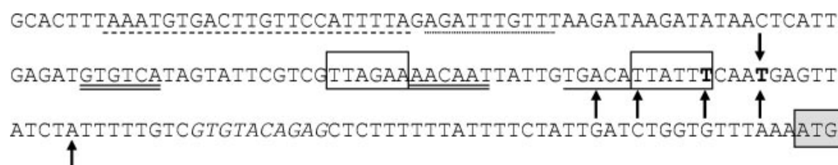


FIG. 1. Nucleotide sequence upstream of *bla*$_{OXA-23}$ indicating transcription start sites (vertical arrows) of *bla*$_{OXA-23}$ in *A. baumannii* (RAM) (↓) and *E. coli*(pRK001) (↑). Major transcription start sites are in bold. The promoters are boxed and double underlined. The sequence (underlined) upstream of TTATTT creates an extended −10 promoter. The *bla*$_{OXA-23}$ start codon is boxed and shaded, and the left inverted repeat of IS*Aba-1* is in italics. The CAP site and UP element half site are underlined with dashed and dotted lines, respectively.

*richia coli* to utilize promoters containing half or full UP-element sites. Mol. Microbiol. **55:**250–260.
13. **Weber, H., T. Polen, J. Heuveling, V. F. Wendisch, and R. Hengge.** 2005. Genome-wide analysis of the general stress response network in *Escherichia coli*: $\sigma^S$-dependent genes, promoters, and sigma factor selectivity. J. Bacteriol. **187:**1591–1603.

**Heidi Segal**
**Rachael K. Jacobson**
**Seike Garny**
**Colleen M. Bamford**
**B. Gay Elisha***
*Division of Medical Microbiology*
*National Health Laboratory Service*
*University of Cape Town*
*Anzio Road, Observatory 7925*
*Cape Town, South Africa*

*Phone: 27 21 4066378
Fax: 27 21 4066129
E-mail: gay.elisha@uct.ac.za

▽ Published ahead of print on 4 June 2007.