

Nucleotide sequence of β-lactamase regulatory genes from staphylococcal plasmid pI258

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Plasmid-encoded penicillinase or β-lactamase (EC 3.5.2.6.), BlaZ, from *Staphylococcus aureus* has been well characterized both enzymologically and genetically (1, 2, 3). It is a small, single-chain exoprotein. β-Lactamase synthesis is inducible by a variety of β-lactam compounds and its regulation in *S.aureus* is similar to that in *B.licheniformis*, including a classical repressor, BlaI, and a positive activator, BlaR1, both plasmid-coded, plus a third, chromosomally located gene, *blaR2* (4). Here we report the nucleotide sequence of the *bla* regulatory region encoded by the plasmid pI258. Plasmid DNA (pI258) was isolated from strain RN11 as described (5). The double-standed DNA sequence of a three kb segment including the *bla* control region was determined using synthetic oligonucleotide primers.

Analysis of the DNA sequence in the *bla* control region (GenBank accession number M62650) revealed two tandem open reading frames of 1755 nt. (ORF-1) and 378 nt. (ORF-2), which overlap by 8 nucleotides (Figure 1). McLaughlin *et al.* (6) have previously published the sequence of the *bla* promoter, including the 7 N-terminal codons of our ORF-1. Their sequence lacks the A at position 177. The basis for this difference is unknown. ORFs 1 and 2 are transcribed in the opposite direction from *blaZ*. Since there are only about 100 bp between the start codons for *blaZ* and ORF-1, it is likely that their promoter sequences overlap. A 55 nt inverted repeat sequence occupies half of this region, including the (*blaZ*) promoter, and has been proposed as the regulatory target (operator) (6). This arrangement is similar to that of the *B.licheniformis blaI* and of the *E.coli* genes, *trpR*, *tetR* and *lexA* (all of which autoregulate their own expression), suggesting that *S.aureus blaI* may be autoregulated as well.

The deduced amino acid sequences of the two ORFs were used to search the GENBANK database using the FASTP program (7). This search revealed that the predicted ORF-1 protein (585 aa) has 26% identity with the *blaR1* protein (601 aa) of *B.licheniformis* (the signal transducer) (8) and 29% identity, in the C-terminal region of 206 amino acids, with the class D β-lactamase (OXA-2) of *Salmonella typhimurium* (9). This region is likely to contain the β-lactam binding site.

ORF-1 protein has a hydrophobicity profile similar to that of the membrane protein of *B. licheniformis*, *BlaR1*, and is probably also an integral membrane protein. Each protein has five predicted trans-membrane segments and a typical hydrophilic C-terminal penicillin binding region that presumably lies external to the membrane. The C-terminal region contains the tetrad, STYK (residues 389 to 392), which is conserved among penicillin binding proteins and includes the active site serine that is bound by penicillins. It also contains the triad KTG (residues 526 to 528), similarly conserved among penicillin binding proteins and essential for their activity (10).

The putative ORF-2 protein (126 aa) shares 38% identity with the 128 aa *B.licheniformis* repressor, BlaI (11). The secondary structure of the ORF-2 protein, predicted using EuGene/SAM software (Molecular Biology Information Resource, Baylor College of Medicine), showed a classical DNA-binding motif, helix-turn-helix, often associated with repressor proteins within residues 80–100 region.

In summary, the *bla* genes comprise a homologous cluster in *B.licheniformis* (12) and *S.aureus*, except that the order of the two regulatory genes is reversed in *S.aureus* (Figure 1).

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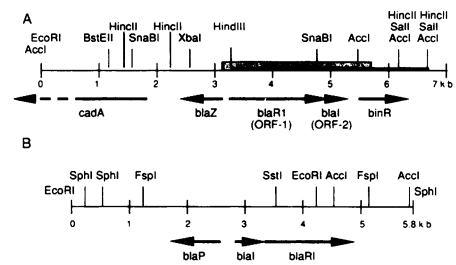

Figure 1. Location of the *blaR1* and *blaI* genes in staphylococcal plasmid pI258 (A), and in the chromosome of *B.licheniformis* (12) (B). The top line shows the restriction sites deposited in GenBank. The DNA sequence is indicated by a cross-hatched box. The sequence cloned is indicated by the bold line. The position of genes are shown with arrows. Kbp, kilobases.