Nucleotide Sequence of an OXA-2 β -Lactamase Gene from the R-Plasmid R1767 Derived Plasmid pBP11 and Comparison to Closely Related Resistance Determinants Found in R46 and Tn2603

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Plasmid pBP11 contains a sequence homologous to Tn21-like element Tn2410 encoding dihydropteroate synthetase and β -lactamase OXA-2. The nucleotide sequence of a 1.5 kb segment of this region has been determined including the *bla* gene. It reveals strong sequence homology with the OXA-2 operon of plasmid R46. The implications of an additional 319 bp segment in pBP11 for the different evolution of R46/pKM101 and pBP11 are discussed.

INTRODUCTION

Resistance to ampicillin, penicillin and other β -lactam antibiotics can be mediated by different enzymes. The majority of these β -lactamases are encoded by plasmids (Foster, 1983), some of which carry the *bla* genes on transposable elements, favouring their dissemination in bacterial hosts. Some of the different β -lactamase genes are well characterized. Ouellette *et al.* (1987) have sequenced the OXA-1 β -lactamase gene (*oxa1*) from Tn2603, an element closely related to Tn21 (Tanaka *et al.*, 1983). Furthermore, Dale *et al.* (1985) and Hall & Vockler (1987) have determined the DNA sequence of the OXA-2 β -lactamase (*oxa2*) expressed by resistance plasmid R46, originally found in *Salmonella typhimurium* (Anderson & Datta, 1965). In this replicon the *bla* gene is located adjacent to *aadA* (Sm^r/Sp^r) and *sul* (Su^r), a segment revealing strong homology to Tn21 and related transposons in its restriction patterns and sequence (Hall & Vockler, 1987). Plasmid pKM101 is a deletion derivative of R46 which has lost the Su^r and Sm^r/Sp^r determinants (Hall & Vockler, 1987).

The conjugative R plasmid R1767 was also derived from a clinical isolate of *S. typhimurium* (Richmond & Wiedemann, 1974). It too mediates resistance to ampicillin by an OXA-2 β -lactamase. A derivative of this plasmid is pBP11 (Tra^-) (Schmidt *et al.*, 1982), a 26.4 kb replicon harbouring its *oxa2* and *sul* resistance determinants on a 6.8 kb duplication. The central region of pBP11 comprising *sul*, *oxa* and *res* is totally homologous to the corresponding part of Tn2410 (Kratz *et al.*, 1983*a, b*). In this paper the nucleotide sequence of its OXA-2 β -lactamase gene and flanking regions are presented. The relationship of the gene and its sequence organization to other *oxa* determinants of R46, Tn2603 and to Tn21 is discussed.

METHODS

Bacterial strains and plasmids. *Escherichia coli* strain RRIAM15 (Rüther, 1982) was used as a host for plasmids. Plasmid pUC19 (Norrander *et al.*, 1984) served as a vector for cloning DNA fragments and mediated resistance to ampicillin. Plasmid pBP11 (Schmidt *et al.*, 1982) served as source for the OXA-2 gene from R1767.

Culture conditions. Growth media and testing for antibiotic sensitivity were as described by van Treeck *et al.* (1981).

DNA techniques. Preparation of plasmid DNA, restriction analysis, molecular cloning and transformation of DNA was done as described previously (Schmidt *et al.*, 1982).

Nucleotide sequence determination. DNA sequencing was done by the method of Maxam & Gilbert (1977) with some modifications as given by Rüther (1982). The 5'-ends of DNA fragments to be sequenced were labelled by treatment with alkaline phosphatase followed by incubation with polynucleotide kinase and [γ - 32 P]ATP (Maxam & Gilbert, 1977). The 3'-ends were labelled by filling in 5'-protruding ends using Klenow fragment of *E. coli* DNA polymerase I and appropriately labelled [α - 32 P]dNTPs (Maniatis *et al.*, 1982). Prior to base specific sequencing reactions, labelled fragments were purified by preparative agarose gel (1.4%) electrophoresis.

Computer analysis. The programs of Devereux & Haeblerli (UWGCG) from the University of Wisconsin (Devereux *et al.*, 1984) were used to analyse DNA and amino acid sequence, to compare sequences for homologies and to search for transcriptional and translational signals.

RESULTS AND DISCUSSION

To determine the nucleotide sequence of the OXA-2 β -lactamase gene from R1767, a 2.8 kb *Hind*III/*Bam*HI fragment containing the resistance gene from pBP11 (Schmidt *et al.*, 1982) was cloned into pUC19. Using the restriction enzyme sites given in Fig. 1, smaller parts from this fragment were subcloned into the pUC vector before sequencing. In addition *Bal*31 deletions from the 2.8 kb fragment were generated, cloned into pUC19 and sequenced. Using all these clones we have determined the DNA sequence given in Fig. 1. The sequence comprises the region coding for OXA-2 β -lactamase and its flanking region.

The results of sequence comparisons to other *bla* genes are given in Fig. 2. The coding sequence of pBP11 (bases 221–1048) is totally homologous to the corresponding DNA sequences published by Dale *et al.* (1985) and Hall & Vockler (1987), revealing that pBP11 contains a *bla* gene identical with that of plasmid R46. As given in Fig. 2, there is no significant homology to the DNA sequence of the OXA-1 β -lactamase published by Ouellette *et al.* (1987). Further DNA sequence homology was found concerning bases 1–201 and 1398–1519 given in Fig. 1. These parts of our sequence resemble the sequences flanking the *aadA* gene of Tn21 (Hollingshead & Vapnek, 1985) (Fig. 2). The only difference between these two sequences is the presence of seven additional bases in the Tn21 region. The extra bases of Tn21 are given in Fig. 1 (inserts at position 65, 76 and 85). The DNA homology was expected because Tn21 is the presumed ancestor of the R1767-derived transposon Tn2410 (Kratz *et al.*, 1983*b*). pBP11 represents the whole antibiotic-resistance region from Tn2410 (Kratz *et al.*, 1983*a*). As can be seen in Fig. 2, some regions of the other presented structures also show a strong homology to the DNA sequence from Tn21. This is not surprising since R46 and Tn2603 are closely related to the Tn21 structure (Hall & Vockler, 1987; Ouellette *et al.*, 1987). Both R46 and Tn2603 harbour a β -lactamase gene adjacent to *aadA* due to an insertion event whereas pBP11 is supposed to represent a substitution derivative of the Tn21 structure (Kratz *et al.*, 1983*b*). In all structures given in Fig. 2 the *oxa* genes are located on the 5' end of *aadA*. The DNA sequences of these regions in Tn2603, R46, pKM101 and pBP11 show complete homology with the DNA sequence published by Hollingshead & Vapnek (1985). In R46 and Tn2603 the homology with this sequence continues on the 3' side of *oxa2* corresponding with the initiation signal sequence of *aadA*. However, pBP11 provides an altered sequence organization. It lacks the *aadA* resistance gene. The homology with Tn21 continues at the 3' end of *aadA* (from position 1398 in Fig. 1). From these sequence data it can be seen that pBP11 contains an OXA-2 gene totally substituting the *aadA* coding region. However, the *aadA* substitution in pBP11 does not comprise only the OXA-2 gene but includes an additional DNA segment of 319 nucleotides. Searching for open reading frames in this region revealed only three very short polypeptides. The first one would use the start codon ATG at position 1109 and the stop codon TAG at position 1181. The resulting polypeptide would consist of 24 amino acids. The next possible coding region uses the complementary strand and extends from base 1413 to base 1299. This would result in a 30 amino acid polypeptide. The third protein could be encoded from the complementary strand of bases 1370 to 1247 and the polypeptide would be 41 amino acids long. All three proposed proteins would be very small. The amino acid sequences of these possible proteins showed no detectable homology with any of the protein sequences of the Protein Identification Resource (PIR) database from the National Biomedical Research Foundation. A comparison of the nucleotide sequence of the unique segment with the National Institutes of Health GenBank database

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ACGCAACTGGTCCAGAACCTTGACCGAAGCGACGGTGGTAACGGCGCAGCTGGCGGTTTTCATCGCTTGTTATGACTGTTTTTTGTATAGTCTATGTCCTCGGGCATCCAAAGCAGCAAGC 120
CGGTTACGCCCTGGGTGCGATGTTTTCATGATGTTATGGACGACCAACGATGTTTACGAGCAGGCGCCTTAAACAAAGTTTGGGCATTAAGGAAAAGTTAATGGCAATCCGAATCTTCGC 240
H
GATACTTTTCTCCATTTTCTCTGGCACTTTCGGCAGTGGCCCAAGACGGCAGCTAGAACGTTCTGACTGGAGGAAGTTTTTCAGCCAAATTTCAAGCCAAAGGCACGATAGTTGTGGC 360
I L F S I F S L A T F A H A Q E G T L E R S D W R K F F S E F Q A K G T I V V A
AGCAGACGCCAAGCGGATCGTCCATGTTTGTGATCTCTGCGATCGAAGAACGCTACTCGCGTCGATCGCAATTCAGATACCTCATACACTTTTTCGCACTTTCGATCGAGCGC 480
D E R Q A D R A M L V F D P V R S K K R Y S P A S T F K I P H T L F A L D A G A
TGTTCTGATCAGTTCACAGATTTTTCGATGGGACGCGTTTACAGGGGCTTTCAGGCCCAATCAAGACCAAGATTTTCGATCAGCAATTCGGAATTTCTACTGTTTGGGTGATGAGCT 600
V R D E F Q I F R W D G V N R G F A G H N Q D Q D L R S A M R N S T V W V Y E L
ATTGCAAGGAATTTGTGATGACAAAGCTCGCGCTATTTCAAGAAATCGCACTATGGCAAGCCGATCTTCGACAGTAATGGCGATTAATGATAGAGGAGCGCTTGCATCTC 720
F A K E I G D D K A R R Y L K K I D Y G N A D P S T S N G D Y W I E G S L A I S
GGCGAGGAGCAATTCATTTCTCAGGAAGCTTCTATCGTAACGAGCTGCGCTTTCGGGTAGAACATCAGCGCTTGGTCAAGGATCTCATGATTTGGAAGCCGCGTCCCACTGGATACT 840
A Q E Q I A F L R K L Y R N E L P F R V E H Q R L V K D L M I V E A G R N W I L
CGGTGCAAGACGGGCTGGGAGCGGTATGGTGGTAGGATGGTGGTGGCGACTGGCTGCTATTTCTCGCACTGAATATTGATAGCCCAACAGAGATGGATGATCTTTT 960
R A K T G W E G R M G W W V G W V E W P T G S V F F A L N I D T P N R M D D L F
CAAGAGGAGGCAATCGTGGGGCAATCTTCGCTCTATTGAAGCGTTTACCGCCCAACCGGCGAGTCACTCGGAGCGCTGCGCGATAAACCGCGAGCGCGGTTACTTCAACGTTAGT 1080
K R E A I V R A I L R S I E A L P P N P A V N S D A A R *
ACCACTGAACCCCTCTTATTTCCGCCATGTTTATTCAACGGCATTCAGTTTCTCAACGGCTGTCAGCGCTGGGTTTCCGCTTCTCGGGCTTACGCTGCTTACGCTGCTTT 1200
GTGCTCTTTTGGCCTCTGGCCCTTGTGTAGCAAGCGGAGCAGCTATTTTTTTCGTAGTGTCTGCGCGCTCGGTGGCACCCTGCTTTCGCAAGTTAGCGCGCTGCGCAAGTTACGG 1320
TTATCCGTTTGGCTCTTACATTTTCGGTCAAGCCGACCGGCATTTCTGCGGTGGCTTACCTCGCCCGTTAGATGCACTAAGCACATAATGCTTCACAGCCAACTATCAGGTC 1440
AAGTCTGCTTTTATTTTAAAGCGTGCAATAAAGCCCTACACAAATTTGGGAGATATATCATGAAGGCTGGCTTTT 1519

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Fig. 1. DNA sequence of the OXA-2 β -lactamase gene and adjacent regions from plasmid pBP11. The deduced amino acid sequence of the enzyme is given below the corresponding DNA region. DNA homology to the *aadA* gene sequence published by Hollingshead & Vapnek (1985) comprises bases 1–201 and 1398–1519. Additional nucleotides of the *aadA* sequence are marked on top of our sequence. The underlined bases represent the sequence with no corresponding regions in the GenBank database. Selected restriction sites are marked on top of the recognition sequence (E, *EcoRI*; H, *HindIII*; Hi, *HinfI*; X, *XhoII*).

Fig. 2. Schematic presentation of the organization of selected β -lactamase-gene-harboring structures. Tn21 is from Hollingshead & Vapnek (1985), pKM101 from Hall & Vockler (1987), R46 from Dale *et al.* (1985), and Tn2603 from Ouellette *et al.* (1987). The enumeration (small numbers) is the same as given in the corresponding publications. The broken line in Tn21 indicates the region of insertion/substitution. Coding regions are marked by bars; a black bar for *aadA*, a shaded bar for *oxa2* and a broken bar for *oxa1*. Mere horizontal lines represent regions of homology with the Tn21 backbone, whereas regions flanked by dotted lines indicate unique sequences.

provided no significant homology with any of the sequences. Both the function and origin of this part of the plasmid pBP11 need further clarification. The presence of the additional 319 bp segment at the 3' end of *oxa* in pBP11 supposes an *oxa2* integration event in pBP11 and Tn2410 differing from that in plasmid R46. While plasmid R46 is considered to be a predecessor of pKM101 (Hall, 1987) no predecessor is known for pBP11. Both pBP11 and pKM101 are spectinomycin sensitive due to different deletion events. IS46 is responsible for the deletion in pKM101 (Hall, 1987) leaving parts of the N-terminus of *aadA* in pKM101 (Fig. 2). In pBP11 and Tn2410 the complete *aadA* gene is deleted. Thus it is most likely that pBP11 and Tn2410 have evolved from Tn21 by insertion of *oxa2* plus additional sequences (unique segment of 319 bp) followed by deletion of the *aadA* gene.

So far the presented data imply the parallel development of plasmids pBP11 and pKM101, providing an example of a convergent evolution.

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