FEMS Microbiology Letters 65 (1989) 241–246
Published by Elsevier

FEMSLE 03771

Nucleotide sequence of the gene encoding the active-site serine β-lactamase from *Actinomadura* R39

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Received 29 June 1989
Accepted 18 July 1989

Key words: Extracellular enzymes; M13 sequencing; Protein primary structure; Actinomyces codon usage

1. SUMMARY

The gene encoding the extracellular, active-site serine β-lactamase of *Actinomadura* R39, previously cloned into *Streptomyces lividans*, has the information for the synthesis of a 304 amino acid protein, the amino terminal region of which has the characteristic features of a signal peptide. The *Actinomadura* R39 β-lactamase is another member of the class A β-lactamases. In particular, it shows high homology with the β-lactamase of *Bacillus licheniformis*.

2. INTRODUCTION

In previous studies [1], DNA fragments containing the gene that encodes the extracellular β-lactamase of *Actinomadura* R39 were introduced in *Streptomyces lividans* TK24 via the high-copy number, promoter-probe plasmid pIJ 424. Maximal level of β-lactamase secretion was observed with *Streptomyces lividans* CM3 harbouring the recombinant plasmid pDML150. Surprisingly, the estimated molecular mass of both the wild and the cloned β-lactamases greatly varied, from 38000 to 57000, depending on the procedures used. The DNA segment which in pDML150 encodes the β-lactamase has now been sequenced. The β-lactamase precursor is a 304 amino acid polypeptide.

3. MATERIALS AND METHODS

Plasmid pDML150, the source of the *Actinomadura* R39 β-lactamase gene [1], plasmid pBR322 [2] and *Escherichia coli* D1210 [3] were used. *S. lividans* and *E. coli* were grown in YEME, Luria-Bertani or M9 media as described [1]. Standard DNA recombinant techniques [4,5] were used except that DNA elution from agarose gels was carried out as described in [6]. The DNA fragments were sequenced by established methods [7]. Phages M13mp10, mp18 and mp19 and tg131 were used as subcloning vectors. Zones of base compression due to the high G + C content of the DNA [8] were resolved by using dITP instead of dGTP (Sequenase kit, USB, Cleveland, U.S.A.) or by performing the gel electrophoreses at 70 °C in the thermostated Macrophor (LKB Instrument,

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Uppsala, Sweden). Codon usage was assessed with Staden's program [9] and Fickett's test [10], using the DD-carboxypeptidase encoding gene of *Streptomyces* R61 [11] as reference. Homology searches were made as in [12]. Synthetic oligonucleotides were purchased from Eurogentec (Liège, Belgium).

4. RESULTS

As shown in Fig. 1A, the 1800 base pair (bp) stretch that was introduced in pDML150 between the two hybrid *Bam*HI/*Bgl*II sites contains the gene encoding the β-lactamase of *Actinomadura*

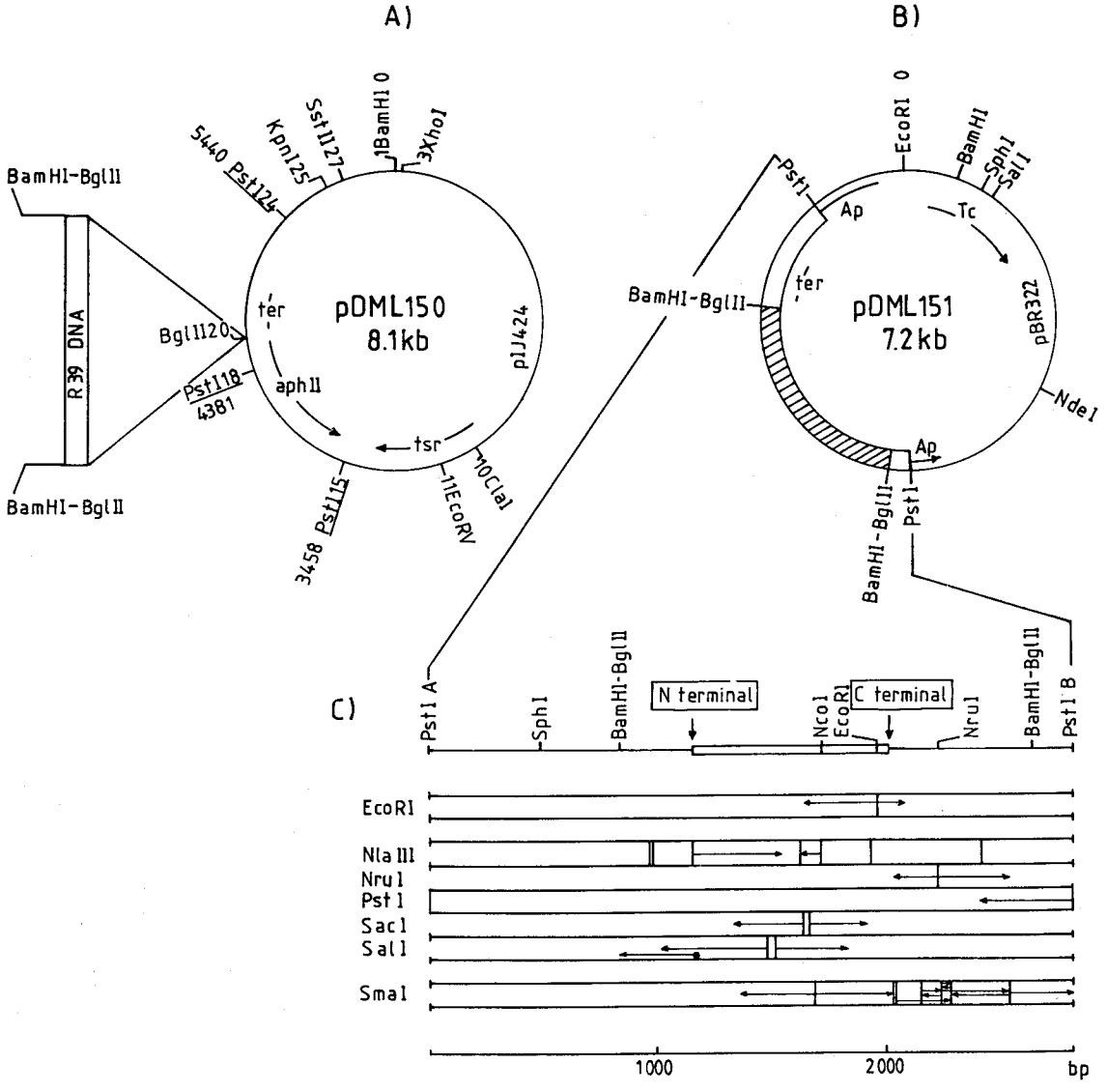

Fig. 1A. pDML150 as derived from pIJ424. The *Pst*I sites used to isolate the β-lactamase-encoding *Actinomadura* R39 DNA insert are base-numbered from the unique *Bam*HI site. 1B. pDML151 recombinant plasmid as derived from pBR322. 1C. Detailed restriction map of the *Pst*I insert and strategy of sequencing. The hatched zone corresponds to the *Actinomadura* R39 DNA. The β-lactamase gene is positioned on the linear map. In the lower part of the figure, the arrows indicate the length of DNA sequenced in each fragment. The black dot shows the position of hybridization of the oligonucleotide synthetic primer (see text).

R39. *Pst*I digestion of both pDML150 and the original pIJ424 generated two common DNA fragments of 4300 bp and 920 bp, respectively, and a third DNA fragment of 1060 bp in the case of pIJ424 and of 2900 bp in the case of pDML150. Hence the 1800 bp insert in pDML150 lacked *pst*I sites. The 2900 bp DNA fragment was isolated.

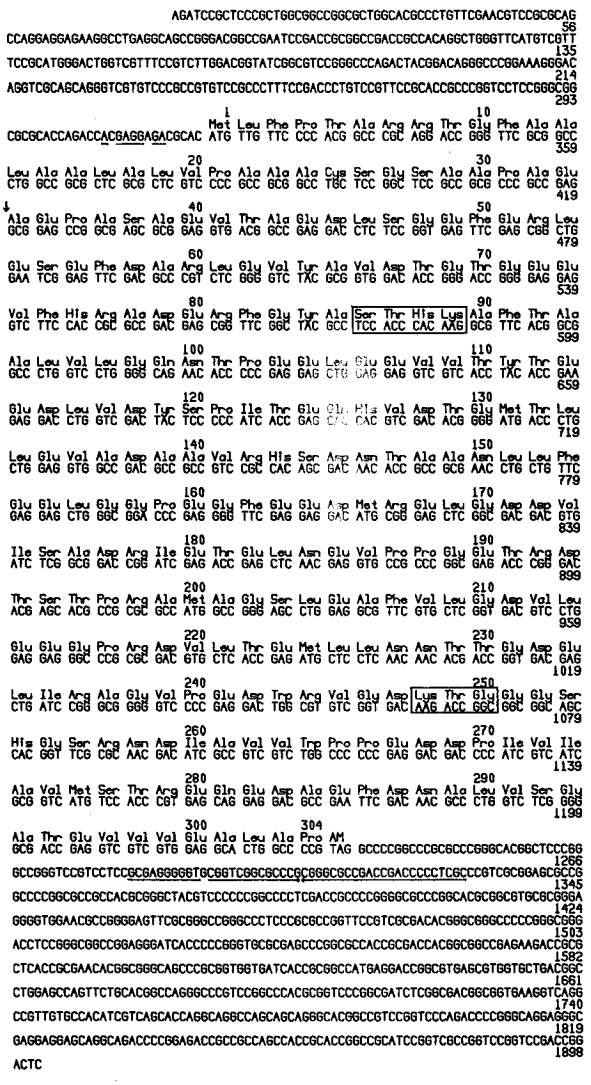

Fig. 2. Nucleotide sequence of the gene encoding the *Actinomadura* R39 β-lactamase and amino acid sequence of the protein precursor. The nucleotides are numbered from the *Bam*HI-*Bgl*II hybrid site. The amino acid numbering refers to the protein precursor. Underlined bases may constitute a ribosome binding site. The amino acids of particular importance are boxed (see text). The vertical arrow indicates a possible processing site and the horizontal arrows a putative transcription terminator signal.

The *Pst*I digest of pDML150 was ligated to pBR322 (previously cut with *Pst*I and treated with alkaline phosphatase) and the ligation mixture was used to transform RecA$^-$ *E. coli* D1210.

Out of 250 Tet^R transformants, only three were Tet^R and Amp^S but each of these contained the desired 2900 bp insert. The corresponding recombinant plasmid was called pDML151 (Fig. 1B). The 2900 bp *Pst*I fragment isolated from both pDML150 and pDML151 gave exactly the same restriction patterns with seven different endonucleases.

As shown in Fig. 1C, the *Pst*IB-*Nla*III 1670 bp stretch was sequenced on both strands for more than 90%. In turn, the *Bam*HI/*Bgl*II-*Nla*III sequence (which escaped analysis because it was too far away from the universal primer in all the possible subclones of the M13 constructs) was sequenced on one strand by subcloning the indicated *Pst*I-*Sal*I fragment and using as primer the synthetic oligonucleotide 5′ = GGTCCTGCGG-GCCGTGGG-3′ (which was complementary to nucleotides 330-347 of the sequence shown in Fig. 2).

5. DISCUSSION AND CONCLUSION

Fig. 2 shows the sequence of that 1902 nucleotide stretch which in the insert originally introduced in pDML150, contained the *Actinomadura* R39 encoding gene. Staden's program and Fickett's test (not shown) permitted identification of an open reading frame (ORF) from nucleotide 321 to nucleotide 1232. The percentage of G + C was 71 globally, and 73, 45 and 96 at the first, second and third position of the triplets. Such a biased codon usage (Table 1) is typical of all known *Streptomyces* genes [13]. It has been also reported for genes of *Micrococcus luteus* [14]. A putative ribosome binding site occurs immediately upstream of the ORF and a putative transcription terminator (i.e. a 46 bp inverted repeat containing only one unpaired base) occurs downstream of the ORF (Fig. 2).

Table 1

Codon usage for the synthesis of the *Actinomadura* R39 β-lactamase

F	TTT	0	S	TCT	0	Y	TAT	0	C	TGT	0
F	TTC	11	S	TCC	6	Y	TAC	4	C	TGC	1
L	TTA	0	S	TCA	0	*	TAA	0	*	TGA	0
L	TTG	1	S	TCG	4	*	TAG	0	W	TGG	2
L	CTT	0	P	CCT	0	H	CAT	0	R	CGT	3
L	CTC	10	P	CCC	11	H	CAC	5	R	CGC	6
L	CTA	0	P	CCA	0	Q	CAA	0	R	CGA	0
L	CTG	16	P	CCG	5	Q	CAG	3	R	CGG	6
I	ATT	0	T	ACT	0	N	AAT	0	S	AGT	0
I	ATC	7	T	ACC	17	N	AAC	8	S	AGC	5
I	ATA	0	T	ACA	0	K	AAA	0	R	AGA	0
M	ATG	6	T	ACG	7	K	AAG	2	R	AGG	1
V	GTT	0	A	GCT	0	D	GAT	0	G	GGT	5
V	GTC	19	A	GCC	22	D	GAC	24	G	GGC	9
V	GTA	0	A	GCA	1	E	GAA	3	G	GGA	1
V	GTG	8	A	GCG	17	E	GAG	38	G	GGG	10

TOTAL CODONS = 304.

Table 2

Amino acid composition of the *Actinomadura* R39 β-lactamase as determined from the wild type (a) and cloned (b) proteins and as deduced from the nucleotide sequence of the gene (c)

Amino acid	% Residues in number		
	(a)	(b)	(c)
Lys	1.6	1.6	0.74
His	1.6	1.4	1.8
Arg	5.6	3	5.2
Trp	3.2	2	0.74
Asx	11.2	13.8	11.8
Thr	6.4	7.8	8.11
Ser	4	4.6	4.8
Glx	14.4	15.8	15.87
Pro	4.8	5.6	4.8
Gly	9.6	11	8.5
Ala	10.4	7.8	10.3
1/2 Cys	1.6	0.6	0
Val	8	10	9.6
Met	1.6	0.8	1.8
Ile	2.4	2.4	2.6
Leu	8.8	8.4	8.5
Tyr	1.6	1	1.5
Phe	3.2	2.6	3.3
	100	100.2	99.9

(a) ref. [15]; (b) ref. [16].

The ORF has the information for the synthesis of a 304 amino acid protein, the amino terminal region of which has the characteristic features of a signal peptide, with two arginine residues occurring at positions 7 and 8 and followed by a Gly10-Val20 highly hydrophobic stretch. The exact site of action of the leader peptidase is not known. The putative Ala34-Pro304 extracellular protein has a 29270 molecular mass. Its amino acid composition is in good agreement with that experimentally obtained for the wild and cloned β-lactamases [1] (Table 2).

```
  SQPAEKNEKT EMKDDFAKLE EQFDAKLGIF ALDTGTNRTV AYRPDERFAF ASTIKALTVG
             *  **   *** **  * ****   *   * ****   *** ** *
  AEPASAEVTAE DLSGEFERLE SEFDARLGVY AVDTGTGEEV FHRADERFGY ASTHKAFTAA
34                                                                94

  VLLQQKSIED LNQRITVTRD DLVNYNPITE KHVDTGMTLK ELADASLRYS DNAAQNLILK
    * *   *  *     ***   *** * **** ********  * ***  * * ** * **
  LVLGQNTPEE LEEVVTYTEE DLVDYSPITE QHVDTGMILL EVADAAVRHS DNTAANLLFE
95                                                               144

  QIGGPESLKK ELRKIGDEVT NPERFEPELN EVNPGETQDT STARALVTSL RAFALEDKLP
    ****     *  ** *     * * *** ** **** ** ** **   **  ** * * * *
  ELGGPEGFEE DMRELGDDVI SADRIETELN EVPPGETRDT STPRAMAGSL EAFVLGDVLE
145                                                              194

  SEKRELLIDW MKRNTTGDAL IRAGVPDGWE VADKTGAASY GTRNDIAIIW PPKGDPVVLA
    *  *      ***** * ****** *  * **** *  * ***** * **  ** * *
  EGPRDVLTEM LLNNTTGDEL IRAGVPEDWR VGDKTGGGSH GSRNDIAVVW PPEDDPIVIA
195                                                              244

  VLSSRDKKDA KYDDKLIAEA TKVVMKALNM NGK
  * * *  **  *  *  * * **  **
  VMSTREQEDA EFDNALVSGA TEVVVFALAP
245                304
```

Fig. 3. Alignment of the β-lactamases of *Bacillus licheniformis* [17] and *Actinomadura* R29. Strict identities are marked by asterisks. The two marks (see text) are underlined.

The *Actinomadura* R39 β-lactamase is another member of the class A β-lactamases. In particular, it has an overall 50% homology and shares 138 identities with the β-lactamase of *Bacillus licheniformis* (Fig. 3) though the G + C contents of the corresponding genes are 71% versus 47%. Note that unlike the other two Actinomycetes β-lactamases of known primary structure [12,18], no insertions or gaps are needed to obtain optimal match. The *Actinomadura* R39 β-lactamase has the two calibration marks found in all the serine, penicillin-recognizing enzymes (β-lactamases and penicillin-binding proteins [19]). Using the amino acid numbering of the precursor, one mark is the active-site serine tetrad Ser86XXLys and the other is the tryad Lys248ThrGly which is known to be also part of the enzyme active site [20].

ACKNOWLEDGEMENTS

We thank Drs. D.A. Hopwood, T. Kieser and M.J. Bibb from the John Innes Institute (Norwich, U.K.) and Dr. J.A. Martial from the Central Laboratory of Genetic Engineering (University of Liège, Belgium) for their generous gifts of strains and plasmids, and Drs. G. Dive and B. Joris (from this laboratory) for their help in computer utilization.

This work was supported in part by the Fonds de la Recherche Scientifique Médicale (contract n° 3.4507.83), an Action concertée with the Belgian Government (convention 86/91-90), the Fonds de Recherche de la Faculté de Médicine ULg and a contract with the EEC (BAP-0197-B). JD is Research Associate at the National Fund for Scientific Research (Belgium).

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