The phototrophic bacterium *Rhodopseudomonas capsulata* sp108 encodes an indigenous class A β -lactamase

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The nucleotide sequence of a 2.37 kb DNA fragment derived from cloning a total DNA digest of *Rhodopseudomonas capsulata* sp108 was determined. The DNA codes for a β -lactamase, a protein showing sequence similarity to the ampR protein of *Enterobacter cloacae* and an unidentified open reading frame. Hybridization experiments with a probe carrying DNA from within the β -lactamase gene suggests a chromosomal location for the coding sequences in strain sp108 and in sp109, a penicillin-sensitive revertant of sp108 in which the enzyme is not inducible. A protein-sequence comparison of the deduced amino acid sequence of the *Rps. capsulata* β -lactamase indicates that it is a Class A enzyme and that its sequence can be aligned with those of the characterized β -lactamases from *Staphylococcus aureus*, *Bacillus licheniformis* and the *Escherichia coli* plasmid (R-TEM enzyme), with only a few insertions or deletions. The corresponding DNA sequence is, however, characteristically rhodopseudomonad, suggesting that it is not a recently transposed gene.

INTRODUCTION

Drug-resistant determinants coding for β -lactamases are widely distributed in bacteria. Several structural classes of β -lactamases are known (Ambler, 1980; Dale *et al.*, 1985), but the commonest appears to be the Class A, which has been recognized in both Gram-positive and Gram-negative bacteria. Class A β -lactamase genes have frequently been located on transmissible plasmids in Gram-negative bacteria (Datta & Kontomichalou, 1965; Ambler & Scott, 1978), but a chromosomal gene which cross-hybridizes with the commonest TEM-type *Escherichia coli* gene has been found in *Klebsiella pneumoniae* (Arakawa *et al.*, 1986). The three-dimensional structures of some Class A enzymes have been determined (Dideberg *et al.* 1987; Herzberg & Moulton, 1987). They are all of M_r about 27000 and show so much similarity throughout their primary sequences that they may be homologous and, hence, must have similar three-dimensional structures. It has been suggested (Tipper & Strominger, 1965; Waxman & Strominger, 1983) that such β -lactamases may have arisen from a gene for a penicillin-binding target enzyme, but as yet the primary sequence and three-dimensional-structural evidence (Kelly *et al.*, 1986) is not conclusive.

Most isolates of Rhodospirillaceae are sensitive to penicillin, but Weaver *et al.* (1975) found a strain of *Rhodopseudomonas capsulata* that was resistant to high levels of β -lactam antibiotics. This strain, sp108, isolated from ground water on a sewage farm, spontaneously yielded a penicillin-sensitive derivative, sp109, and for

this reason Weaver *et al.* (1975) suspected that the original resistance was due to a β -lactamase. Scahill (1981) confirmed this suggestion and showed that strain sp108 produced an inducible periplasmic β -lactamase with a pI of 4.5; strain sp109 produced a similar enzyme, but higher levels of synthesis were not inducible. He investigated the substrate profile of the enzyme and showed that it had little action against cephalosporins. The rates of hydrolysis of antibiotics relative to that of 100 for benzylpenicillin were: ampicillin, 27; carbenicillin, 25; cloxacillin, 0; cephaloridine, 4.

It seemed worthwhile to characterize this β -lactamase and see if its properties gave any indications of its origin. Phototrophic bacteria like *Rps. capsulata* are normally found in water, an environment unlikely to contain damaging concentrations of fungally produced antibiotics. Such organisms are also unlikely to be exposed to the intentional chemotherapeutic use of penicillins and will not generally compete with non-photosynthetic micro-organisms for nutrients. Nevertheless, there is laboratory evidence that rhodopseudomonads can accept resistance-transfer factors from other Gram-negative bacteria (Sistrom, 1977), so a plasmid origin for the sp108 enzyme seemed a likely hypothesis.

In the present paper we report the sequence of a region of *Rps. capsulata* sp108 DNA that includes an open reading frame (ORF) coding for a class A β -lactamase. Evidence is given that suggests that this piece of DNA is chromosomal, even though strain sp108 contains several distinct plasmids, as do most strains of *Rps. capsulata* (Saunders *et al.*, 1976).

Abbreviation used: ORF, open reading frame.

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EXPERIMENTAL

Materials

Rps. capsulata strains sp108 and sp109 were obtained from Dr. Judy Wall (Department of Microbiology, University of Indiana, Bloomington, IN, USA). Plasmid pSS20 is a recombinant composed of a 5.8 kb DNA fragment from *Rps. capsulata* sp108 cloned into the restriction-endonuclease-*Bam*HI site of pBR322 (Scahill, 1981). DNA polymerase I (Klenow fragment) and DNA ligase were obtained from Boehringer Mannheim. All the restriction endonucleases were purchased from N.B.L. Enzymes, Cramlington, Northd., U.K., and radiolabelled chemicals were from Amersham International.

Preparation and management of nucleic acids

All DNA preparations and recombinant DNA techniques were performed as described by Maniatis *et al.* (1982). DNA fragments were sequenced as recombinants in bacteriophage M13 using the dideoxy-chain-termination method described in detail by Bankier & Barrell (1983).

DNA transfer to nitrocellulose filters and hybridization

DNA was transferred from agarose gels by the method of Southern (1975). After transfer, the filters were incubated in the presence of the probe for 16–24 h at 37 °C in 50% (v/v) formamide/0.1% (w/v) SDS/[600 mM-NaCl/60 mM-sodium citrate, pH 7.0 (4× SSC buffer)]/0.02% (w/v) bovine serum albumin/0.02% (w/v) Ficoll/0.02% (v/v) polyvinylpyrrolidone/denatured calf thymus DNA (50 µg/ml). The filters were washed for 2 h in half-strength SSC buffer containing 0.1% (w/v) SDS, and for another 2 h in plain half-strength SSC buffer. The filters were then dried and exposed to X-ray films. Autoradiographs were developed for 6–16 h at –70 °C.

M13 hybridization probes

The primer-extension method of Hu & Messing (1982) was used to incorporate label into single-stranded template DNA. The primer (PHM 235) (New England Biolabs) is complementary to a region in the 5' side of the insert, so that whereas the M13 vector is made double-stranded, the insert remains unpaired and can be hybridized to related DNA.

M13 Template DNA (5 µl) was mixed with 1 µl of 1 mM-Tris/HCl(pH 8)/6 mM-NaCl/0.66 mM-MgCl₂ (10× Hin buffer) and annealed with 1 µl of primer in a final volume of 10 µl for 60 min at 65 °C. The mixture was cooled to

room temperature (20 °C), and polymerization was initiated by adding 6 µl of water, 3 µl of a mixture of 500 µmol each of dATP, dTTP and dGTP, 10 µCi [α -³²P]dCTP and 1 unit of Klenow enzyme to a final volume of 20 µl. The reaction was allowed to continue for 90 min and was then terminated by the addition of 100 µl of 10 mM EDTA. This preparation was used in the hybridization reaction without any denaturation.

Sequence analysis

The DNA sequences were compiled with the aid of the computer programs DBAUTO and DBUTIL (Staden, 1982) and then analysed for gene-coding features using ANALYSEQ (Staden, 1984). The 'Protein Identification Resource' (George *et al.*, 1986) procedure was carried out using the program FASTP (Lipman & Pearson, 1985). The University of Wisconsin Genetics Computer Group (UWGCG) program PRETTY was used to make amino acid and nucleotide sequence alignments (Devereux *et al.*, 1984).

RESULTS

Subcloning the β -lactamase gene

The β -lactamase gene had been located to a 5.8 kb DNA fragment cloned into the *Bam*HI site of pBR322 (Scahill, 1981). The resulting recombinant plasmid, pSS20, was mapped; the restriction-enzyme sites and approximate sizes of the resulting fragments in the rhodopseudomonad DNA are represented in Fig. 1. The *Sal*I fragment was generated because of a flanking *Sal*I site situated within pBR322 DNA only 275 bp from the *Bam*HI junction site (Sutcliffe, 1978).

E. coli transcription systems do not recognize the gene-expression systems of photosynthetic bacteria (Chory & Kaplan, 1982). Thus λ vectors and expression plasmid vectors were used in cloning the rhodopseudomonad β -lactamase gene.

The *Xho*I- and *Sal*I-derived fragments were ligated with *Sal*I-digested λ 857cl Sam in separate experiments. The two *Sal*I sites on λ -DNA occur 0.6 kb from each other within the *gam* gene (Sanger *et al.*, 1982). This is part of the replaceable non-essential region. As λ -phage can only package DNA that is 80–105% of its genomic size (Weil *et al.*, 1973), prospective inserts must be less than 3 kb. Thus both fragments (2.6 and 2.2 kb) were within the described range.

β -Lactamase-producing phages identified as plaques surrounded by a halo of bacterial cells on ampicillin L/agar plates were only observed with phage DNA

Fig. 1. Physical map of the *Rps. capsulata* 5.8 kb DNA fragment

The continuous line represents *Rps. capsulata* DNA, whereas the broken line represents flanking vector (pBR322) DNA before the *Sal*I site.

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1  GGATCCGAACCTTCTACAAAGCCCATCGGCTATCTCCGCGACGGGGCCAGGAGCCACTCTCCAGCCCTCAACCCGCTGCGCGGAAAT
91  CGACACTCCCTTACTCTGGCTGATGTGAAGGGCGGGAAGGGACCTTCCGCGGCTTGGGTGAAAGTCCGGAAGGGCGCTCTCTGCTG
181  GGCAGACCCCACTCGCTTGGGGGGCAGCAAGCTGTGCCGCGCAACTCTGCTTCGAGCCCTTTCGAGCCCTTCTGTGTGGTTTGGGCTT
271  CATGTGTCATGACGGGCATCCAAGTGGGTGCCATGTCCGCGGCGCTTAGCGGSCACGGCAGGCTTGGGGGGGGGTGACAAGAAT
361  TGGATGCTTACTCAGCGCTCTGCGCGGTTCAGCCAAACGCGAGAAGCTGCTCATCGCTGATGTGGGGGGCGGTTCGACGGCCAGGCGAGAT
    * E A S Q G T L W R S F T S M A S T A P R D S P W A L Y
451  AGTAACGTCGACAGAACTGTGACCGCGAAAGGCTGTGCCAAGCGGCCCTGCGGATGTAGCTCTCGAACATGGAGATGGCAGCAGGG
    Y R G V S V T V G F P Q A L R G Q A I Y S E F M S I P L L A
541  CGACGCGCGCGCTGAGGTTCGACGCTCTGCCAAAGCCACCGAGCTGTCAAAGACCGCGCGGTGACAGGGGGCAAGGCCACGCTCGGG
    V G A G S T A L E A L A V S S D F V P G T V P P C P V G A A
631  CCTCAAACCGCCCGCCATTCGCGCGCTTCGGTAGCTGCGCAGAACGGTTACTTCCCGAGGTCGAGGGATGCAGCAGCCGTGACGCGA
    E F W G P W E A S R Y S R L L T V Q G L D S P H L L R S A L

                                                                    [URF1] M
721  GGGCGGCGCGCAGAGCGCGCCATCGGCGCTCTGCCAGTGGGATCGCATCGTGTCTGTCCAGCCTCCAGTGGCGAAACGGATCGCA
    G P A C L P A M P A E A L P I A D H G T W G G T G F R I A M
    S S P S R R I S T R L L L V E I L R S I W G W A C R N A S S
811  TGTGAGCCCCCTCGCGCAGGATCTCCACCGGTTGTGTGTGGTTCGAGATCTCAGGTTCAGTCTGGGGATGGGCTGTTCGGAACCGCTCGA
    D L G E R L I E V R N N N T S I R L D I Q P H A Q R F A E L
    R G I S Q H I A K V V L T P T F S T S M S R R P P R R S S T
901  GCGCGGCATCAGCCAGCACAATCGCGAAGGTCTGTGTGAAGCCAACTTACGACCTCGATGTTCGCGCGCGCGCGGAGACGGTCGAGCA
    R P M L W C M A F T T N V G V K L V E I D R R G G L R D L V
    R D I A S N P C S S T G K S R R P S S V G I S P W E V L R N
991  CCGCGACATCGCGTCGAACCCATGCTCAAGCAAGGAAAGAGCAGGCTCCCTCGTTCGCGGATCAGCCCTGGGAGGTCTCTCAGGA
    R S M A D F G H E L V P F L L R G E D T P I L G Q S T R L F
    S A V P S R S S S R A T W W L T A A W V T R S S I A A L V K
1081  ACAGCGCTGTGCCAGCAGGTCTCGAGCGCGCGACCTGGTGGCTGACGGCAGCCTGGGTGACGCGCAGCTCGATCGCGCGCTTGGTAA
    L A T G L L D E L R A V Q H S V A A Q T V R L E I A A K T F
    L P W R M A T S N T R R A L S G R S G R S M A V P Q L M A G
1171  AGCTGCCCTGGCGCATGGCGACCTCGAATAGCGCGAGGCACTTGAAGCGCAGGTTCGGGGGATCCATGGCAGTACCTCAGCTAATGGCAG
    S G Q R M A V E F V R L A N L P L D P R D M [AmpR]
    H K I C R L M A A I K P K Y T V R M T S C D V A S Q A P L A
1261  GCCATAGCTTGTGCTTGTATGGCTGCGATCAAGCGGAAATATACAGTCCGATGACATCATGTGATGTGCAATCCAGCGCGCACTGG
    [β-lactamase] M R F T A T V L S R V A T G L A L G L S M A
    Q I L K G M V P C G S P L P S C R V S R Q G S L S A C P W P
1351  CGCAGATCTCTGAAGGATGGTCCATCGGTTACCGCTACCGTCTGTTCGCGTGTTCGCGACAGGGCTCGCTCTCGGCTGTTCATGCG
    T A S L A E T P V E A L S E T V A R I E E Q L G A R V G L S
    R P P S P K R L S R R S P K P S P G S R N S S A P A S A S R
1441  CACGGCTCCCTCGCGAAACGCTCTCGAGGCGCTCTCGAAACGCTCGCGCGATCGAGGAACAGCTCGCGCGCGCGGTTCGCTCTC
    L M E T G T G W S W S H R E D E L F L M N S T V K V P V C G
    S W R P A R V G P G L T A R T S F S S *
1531  GCTCATGGAGACCGGCAAGGTTGGTCTGTCTCAGCGGAGGACAGCTTTTCTCATGAACAGCAAGGTCAGGTTCCGGTCTCGGG
    A I L A R W D A G R L S L S D A L P V R K A D L V P Y A P V
1621  CGCCATCTTCGCGGTTCGGACGCGGGCAGGCTGTGCTCTTCGATGCGCTGCGGTTCGCAAGGCGACCTCGTGCCCTACGCGCGGT
    T E T R V G G N M T L D E L C L A A I D M S D N V A A N I L
1711  CACGGAGACGCGGGTTCGCGCAACATGACCTTCAGCAGCTCTGCTCGCGCGATCGACATGAGCGACAATGTGGCGCGAATCTCT
    I G H L G G P E A V T Q F F R S V G D P T S R L D R I E P K
1801  GATGGGCATCTCGGGGGCGGAGGCGGTGACGAGTTCTTCGCGAGCTCGCGGACCGGACGCGCTCTCGACCGCATCGAGCCAA
    L N D F A S G D E R D T T S P A A M S E T L R A L L L G D V
1891  GCTGAACGACTTCGCTTCTGGAGACGAGCGGACACGAGCGCGCGCCATGTTCGAGACGCTGCGAGCGCTGCTGCTGGGCGAGCT
    L S P E A R G K L A E W M R H G G V T G A L L R A E A E D A
1981  GCTGTCTTCGCGAGGCGCGCGGAGCTGGCGGAGTGGATGCGCACGCGCGGTGACCGCGCATTTGCTGCGCGCGAGGCGGAGGCGC
    W L I L D K S G S G S H T R N L V A V I Q P E G G A P W I A
2071  CTGGCTGATCTTCGCAAGTGGGCGAGCGGAAGCCACGCGCAACCTCTTCGCGTGTATCCAGCTGAAGGCGGAGCGCGCTGATTCGC
    T M F I S D T D A E F E V R N E A L K D L G R A V V A V V R
2161  GACCATGTTCATCTCGGATACGACGCGGAGTTGAGGTTTCGCAACGAGGCGCTCAAAGATCTGGGTAGGGGCGGTTCGCGGTGTTCG
    E *
2251  CGAATAGCCTATCCAGGCGCGGCTTCGCTCTGAGAAAGGATGTACTTTGGGCTGCGGAGCAGTGAGACATCATGCCATTTCGCGAGCG
2341  ATGTGTCGCGGTGGGGTGCCTGCAATACCTGGTGCAC

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Fig. 2. Nucleotide sequence of the *Rps. capsulata* β -lactamase gene and flanking DNA

The boxed sequences indicate rhodopseudomonad ribosome binding sites [Shine–Dalgarno sequences]. Amino acid translations of the ORFs have been presented in their one-letter notation.

Fig. 4. Location of the *Rps. capsulata* sp108 β -lactamase gene

Recombinant plasmid carrying the *Rps. capsulata* β -lactamase gene as well as total DNA and native plasmids of the *Rhodopseudomonas* spp. were digested and the fragments were separated in 0.75% agarose gels (a). The Southern transfer of DNA from gel (a) was then hybridized to a DNA probe from within the β -lactamase gene (b). Lanes: 1, pBR322 digested with *Bam*HI; 2, pSS20 digested with *Bam*HI; 3, pSS20 digested with *Sal*I; 4, *Rps. capsulata* sp108 plasmids undigested; 5, *Rps. capsulata* sp108 plasmids digested with *Bam*HI; 6, *Rps. capsulata* sp108 total DNA digested with *Bam*HI; S, Marker DNA; 8, *Rps. capsulata* sp109 plasmids undigested; 9, *Rps. capsulata* sp109 plasmids digested with *Bam*HI; 10, *Rps. capsulata* sp109 total DNA digested with *Bam*HI. The hybridization of the probe to the 5.8 kb *Bam*HI insert and 2.6 kb *Sal*I fragment of pSS20 as well as to a 5.8 kb DNA band (indicated by an arrow) in tracks 6 and 10 suggests that the β -lactamase is chromosomally mediated. The size markers (S) are derived from a mixture of *Hind*III digest of λ -DNA and *Hae*III digest of ϕ X-phage DNA.

of the ORFs to the *Bacillus licheniformis* 749 β -lactamase protein sequence held in the EMBL Library using the program DIAGON (Staden, 1982). A short sequence resembling Shine & Dalgarno (1974) sequences predicted for rhodopseudomonad genes from 16 S RNA studies, AAGGAA (Gibson *et al.*, 1979), was found 7 pb upstream of the initiation codon of the β -lactamase gene.

The predicted amino acid sequences of ORFs 1 and 3 were compared with the Protein Identification Resources by using the computer program FASTP, and a highly significant similarity was found. ORF 3 has a high level of identity with the *ampR* protein of *Enterobacter cloacae*. Both proteins share a 48.3% identity spread throughout their entire length, with a further 35% being conservative changes (Fig. 3). The *ampR* gene codes for a regulatory protein which has a positive effect on *ampC* expression in the presence of inducing β -lactams (Lindberg *et al.*, 1985; Honoré *et al.*, 1986).

Location of the β -lactamase gene

The original clone of the *Rps. capsulata* sp108 β -lactamase gene was derived from cloning a total genomic digest into λ 781-0 (an *Eco*R1 replacement vector; Murray *et al.*, 1977). The recombinant phage was termed ' λ LS20' (Scahill, 1981). Scahill (1981) had also observed and studied several native plasmids in strains sp108 and sp109. Both had the same range of plasmids and differences in plasmid sizes or in restriction-fragment

patterns generated could not be observed when the strain sp108 was grown in the presence or absence of penicillin. In addition, growth in ethidium bromide did not abolish the penicillin-resistant phenotype.

In the course of these investigations, an M13 hybridization probe made from template carrying an insert from nucleotide 1523 to 1757 (a region within the β -lactamase gene), was used to probe *Bam*HI digests of genomic and plasmid DNA preparations of *Rps. capsulata* sp108 and sp109.

The probe hybridized to bands corresponding to the 5.8 kb *Bam*HI fragments in *Rps. capsulata* sp108 and sp109 genomic tracks (Fig. 4), thus suggesting that the β -lactamase gene is chromosomally mediated.

Characterization of the β -lactamase

(i) **Amino acid sequence alignment.** As the *Rps. capsulata* sp108 β -lactamase sequence was identified using the *B. licheniformis* 749/C β -lactamase sequence, it was evident that this was a member of the class A group (Ambler, 1980). The deduced protein sequence is aligned with some characterized class A enzymes in Fig. 5 by using the Ambler (1980) scheme. Generally, the alignment is very good, suggesting that these sequences are all derived from the same ancestral gene. Many of the invariant residues were retained and all the enzymes are approximately the same size, suggesting that there have been few, if any, insertions or deletions. The invariant

	1				50
Pns:				MKKL	IFLIVIALVL
Bcl:	MI	LKNKRMLKIG	ICVGILGLSI	TSLEAFTGES	LQVEAKEKTG
Blp:		...MKLWFST	LKLKKAQAVL	LFSCVALAGC	ANNQTNASQP
Pne:				..MSIQHFRV	ALIPFFAAFC
Ppa:			MRKFO	CHFLSVPAI	LGCVGICTS
Rcp:			MRFTA	TVLSRVATLG	ALGLSMATAS
Con:		-----	-----	-----	-----
	51				100
Pns:	SACNSNSSHA	KELNDLEKKY	NAHIGVYALD	TRSGKE.VKF	NSDKRFAYAS
Bcl:	QVKHKNOATH	KEFSQLEKKF	NARLGVYALD	TGTNQT.ISY	RPNERFAFAS
Blp:	AEKNEKTEMK	DDFAKLEEQF	DAKLGFALD	TGTNRT.BAY	RPDERFAFAS
Pne:	LPVFAHPETL	VKVKDAEDQL	GARVGYIELD	LNSGKILES	RPEERFPMMS
Ppa:	AYAMDTGILD	LAVTQEETTL	QARVGVAVID	TDSGLTWQ.H	RGDERFPLNS
Rcp:	LAETPVEALS	ETVARIEEQL	GARVGLSLME	TGTGWSWS.H	REDELFMLNS
Con:	-----	-----e---	-a--g-----	-----	-----f---s
	101				150
Pns:	TSKAINSAIL	LEQVPYNKLN	KKVHI..NKD	DIVAYSPILE	KYVGKD.ITL
Bcl:	TYKALAAGVL	LQONISDSL	EV..ITYTKE	DLVDYSPVTE	KHVDTG.MKL
Blp:	TIKALTGVVL	LQOKSIEDLN	QR..ITYTRD	DLVNYNPITE	KHVDTG.MTL
Pne:	TFKVLLCGAV	LSRVDAGQEQ	LGRRIHYSQN	DLVEYSPVTE	KHLTDG.MTV
Ppa:	THKAFSCAAV	LAQADRHKLM	LEQAIPIERT	ALVTYSPVTE	RVPPGGTTL
Rcp:	TVKVPVCGAI	LARWDAGRLS	LSDALPVRKA	DLVPAYPVTE	TRV.GGNMTL
Con:	t-k-----	l-----	-----	--v-y-p--e	-----
	151				200
Pns:	KALIEASMTY	SDNTANNKII	KEIGGIKKVR	ORLKELGDKV	TNPVRYEIEL
Bcl:	GEIAEAAVRS	SDNTAGNILF	NKIGGPKGYE	KALRHMGDRI	TMSNRFETEL
Blp:	KELADASLRY	SDNAAQNLIL	KJIGGPESLK	KELRKIGDEV	TNPERFEPEL
Pne:	RELCSAAITM	SDNTAANLLL	TTIGGPKELT	AFLHNMGDHB	TRLDRWEPEL
Ppa:	RELCAAASVI	SDNTAANLAL	DAIGGARTFT	AFMRSIGDDK	TRLDRREPEL
Rcp:	DELCLAAIDM	SDNVAANILI	GHLGGPEAVT	QFFRSVGDPT	SRLDRIEPEL
Con:	----a-----	sdn-a-n---	---gg-----	-----gd--	----r-e--l
	201				250
Pns:	NYSPKSKKD	TSTPAAFGKT	LNKLIANGKL	SKENKKFLLD	LMLNNKSGDT
Bcl:	NEAIPGDIRD	TSTAKAIATN	LKAFTVGNAL	PAEKRKILTE	WMLGNATGDK
Blp:	NEVNPGETQD	TSTARALVTS	LRAFALEDKL	PSEKRELLID	WMKRNTTGDA
Pne:	NEAIPNDERD	TTMPAAMATT	LRKLLTGELL	TLASRQQLID	WMEADKVAGP
Ppa:	NEATPGDARD	TTTPIAAARS	LQTLLLDGVL	SAPARNELTQ	WMLGDQVADA
Rcp:	NDFASGDERD	TTSPAAMSET	LRALLLGDLV	SPEARGLAE	WMRHGGVTGA
Con:	n-----d	t----a----	l-----l	-----l--	-m-----
	251				300
Pns:	LIKDGVPKDY	KVADKSGQAI	TYASRMDAVF	VYPKGQSEPI	VLVIF.TNKD
Bcl:	LIRAGIPTDW	VVGDKSGAG.	SYGTRNDIAV	VWP.PNSAPI	I..VLISSKD
Blp:	LIRAGVPDGW	EVADKTGAA.	SYGTRNDIAI	IWP.PKGDPV	VLAVL.SSRD
Pne:	LLRSALPAGW	FIADKSGAG.	ERGSRGIIAA	LJP.DGKPSR	IVVIY.TDGS
Ppa:	LLRAGLPRDW	QIADKSGAG.	GHGSRSIIAV	VWP.PKRSAP	IVAIYITQTA
Rcp:	LLRAEAEDAW	LILDKSGSGS	HTRNLVAVIQ	PEGGAPWIAT	MFISDTDAEF
Con:	l-----	---dk-g---	-----	-----	-----
	301		327		
Pns:	NKSDKPNDKL	ISSETAKSVMK	EF.....		
Bcl:	EKEAIYNDQL	IAEATKVIVK	GS.....		
Blp:	KKDAKYDDKL	IAEATKVVMK	ALNMNGK		
Pne:	QATMDERNRQ	IAEIGASLIK	HW.....		
Ppa:	ASMSASNQAV	SRIGSALAKA	LQ.....		
Rcp:	EVRNEALKDL	GRAVVAVVRE			
Con:	-----	-----	-----		

Fig. 5. Amino acid sequence alignment of class A β -lactamase

The amino acid sequences are aligned on the system of the Ambler classification. The residues identical in all the sequences are represented in the consensus string. The numbering has been arranged such that the active-site serine residue is numbered '100'. Pns, *Staph. aureus* PC1 (Ambler, 1975); Bcl, *B. cereus* 569/4 (Thatcher, 1975); Blp, *B. licheniformis* 749/C (Ambler & Meadway, 1969); Pne, *E. coli* TEM1 (Sutcliffe, 1978); Ppa, *Ps. aeruginosa* RMS 149 (J. I. Campbell, T. Gibson & R. P. Ambler, unpublished work); Rcp, *Rps. capsulata* sp108 β -lactamase; Con, consensus sequences.

1	E	GAGAACACG	TTGAGGCTAG	GCTGGAGTG	GCGGTGATCG	ATACGGATTC	50
Ppa DNA:		GAGAACACG	TTGAGGCTAG	GCTGGAGTG	GCGGTGATCG	ATACGGATTC	
Rcp DNA:		GAGAACACG	TTGAGGCTAG	GCTGGAGTG	GCGGTGATCG	ATACGGATTC	
Pne DNA:		GAGAACACG	TTGAGGCTAG	GCTGGAGTG	GCGGTGATCG	ATACGGATTC	
Blp DNA:		GAGAACACG	TTGAGGCTAG	GCTGGAGTG	GCGGTGATCG	ATACGGATTC	
Consensus		g a - - - - -	t - - - - -	- - - - -	- - - - -	- - - - -	
51		CGGCTGAGC	TGCGT...	C ATCGTGGCGA	CGAACGCTTC	CCGCTGAACA	100
Ppa DNA:		CGGCTGAGC	TGCGT...	C ATCGTGGCGA	CGAACGCTTC	CCGCTGAACA	
Rcp DNA:		CGGCTGAGC	TGCGT...	C ATCGTGGCGA	CGAACGCTTC	CCGCTGAACA	
Pne DNA:		CGGCTGAGC	TGCGT...	C ATCGTGGCGA	CGAACGCTTC	CCGCTGAACA	
Blp DNA:		CGGCTGAGC	TGCGT...	C ATCGTGGCGA	CGAACGCTTC	CCGCTGAACA	
Consensus		- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	
101	S T K	GACGCTAA	AGCTTTTCC	TGCGGCGGCG	TTCTGGCCCA	GGCCGACCGC	150
Ppa DNA:		GACGCTAA	AGCTTTTCC	TGCGGCGGCG	TTCTGGCCCA	GGCCGACCGC	
Rcp DNA:		GACGCTAA	AGCTTTTCC	TGCGGCGGCG	TTCTGGCCCA	GGCCGACCGC	
Pne DNA:		GACGCTAA	AGCTTTTCC	TGCGGCGGCG	TTCTGGCCCA	GGCCGACCGC	
Blp DNA:		GACGCTAA	AGCTTTTCC	TGCGGCGGCG	TTCTGGCCCA	GGCCGACCGC	
Consensus		- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	
151		GACAAGCTG	ACCTGGAGCA	GGCGATACCG	ATCGAGCGCA	CAGCGCTGCT	200
Ppa DNA:		GACAAGCTG	ACCTGGAGCA	GGCGATACCG	ATCGAGCGCA	CAGCGCTGCT	
Rcp DNA:		GACAAGCTG	ACCTGGAGCA	GGCGATACCG	ATCGAGCGCA	CAGCGCTGCT	
Pne DNA:		GACAAGCTG	ACCTGGAGCA	GGCGATACCG	ATCGAGCGCA	CAGCGCTGCT	
Blp DNA:		GACAAGCTG	ACCTGGAGCA	GGCGATACCG	ATCGAGCGCA	CAGCGCTGCT	
Consensus		- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	
201	Y P E	GACGCTGCA	CCGCTGACG	AAAGGGTGGC	ACCTGGCGGC	CAGCGCTGCT	250
Ppa DNA:		GACGCTGCA	CCGCTGACG	AAAGGGTGGC	ACCTGGCGGC	CAGCGCTGCT	
Rcp DNA:		GACGCTGCA	CCGCTGACG	AAAGGGTGGC	ACCTGGCGGC	CAGCGCTGCT	
Pne DNA:		GACGCTGCA	CCGCTGACG	AAAGGGTGGC	ACCTGGCGGC	CAGCGCTGCT	
Blp DNA:		GACGCTGCA	CCGCTGACG	AAAGGGTGGC	ACCTGGCGGC	CAGCGCTGCT	
Consensus		- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	
251	E	TGCGTACGT	GTGACGGCG	GCGCTCAGTA	TGAGTGGGCA	CAGCGCTGCT	300
Ppa DNA:		TGCGTACGT	GTGACGGCG	GCGCTCAGTA	TGAGTGGGCA	CAGCGCTGCT	
Rcp DNA:		TGCGTACGT	GTGACGGCG	GCGCTCAGTA	TGAGTGGGCA	CAGCGCTGCT	
Pne DNA:		TGCGTACGT	GTGACGGCG	GCGCTCAGTA	TGAGTGGGCA	CAGCGCTGCT	
Blp DNA:		TGCGTACGT	GTGACGGCG	GCGCTCAGTA	TGAGTGGGCA	CAGCGCTGCT	
Consensus		- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	
301	N	TGCGTACGT	GTGACGGCG	GCGCTCAGTA	TGAGTGGGCA	CAGCGCTGCT	350
Ppa DNA:		TGCGTACGT	GTGACGGCG	GCGCTCAGTA	TGAGTGGGCA	CAGCGCTGCT	
Rcp DNA:		TGCGTACGT	GTGACGGCG	GCGCTCAGTA	TGAGTGGGCA	CAGCGCTGCT	
Pne DNA:		TGCGTACGT	GTGACGGCG	GCGCTCAGTA	TGAGTGGGCA	CAGCGCTGCT	
Blp DNA:		TGCGTACGT	GTGACGGCG	GCGCTCAGTA	TGAGTGGGCA	CAGCGCTGCT	
Consensus		- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	
351	G D	TGCGTACGT	GTGACGGCG	GCGCTCAGTA	TGAGTGGGCA	CAGCGCTGCT	400
Ppa DNA:		TGCGTACGT	GTGACGGCG	GCGCTCAGTA	TGAGTGGGCA	CAGCGCTGCT	
Rcp DNA:		TGCGTACGT	GTGACGGCG	GCGCTCAGTA	TGAGTGGGCA	CAGCGCTGCT	
Pne DNA:		TGCGTACGT	GTGACGGCG	GCGCTCAGTA	TGAGTGGGCA	CAGCGCTGCT	
Blp DNA:		TGCGTACGT	GTGACGGCG	GCGCTCAGTA	TGAGTGGGCA	CAGCGCTGCT	
Consensus		- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	

Fig. 6. Nucleotide-sequence alignment of class A β -lactamase genes

These were aligned in the same order as were the amino acid sequences in Fig. 5. The variable leader-peptide sequences were excluded, and the codons for the 43 invariant amino acids have been boxed to highlight the nucleotide polymorphism. Ppa DNA, *Ps. aeruginosa* RMS 149 β -lactamase gene; Rcp DNA, *Rps. capsulata* sp108 β -lactamase gene; Pne DNA, *E. coli* Tem1 gene on pBR322 (Sutcliffe, 1978); Blp DNA, *B. licheniformis* β -lactamase gene (Neugebauer *et al.*, 1981).

active-site serine residue (Cartwright & Coulson, 1980) has been numbered 100 so as to give a stable numbering system without numbers less than one. Although we have not analysed the protein properties of the sp108 β -lactamase reported here, the alignment observed is good evidence that the deduced amino acid sequence is correct.

In addition, examination of the derived amino acid sequence leads to the prediction that the *Rps. capsulata* β -lactamase molecule has a considerable excess of negative charges at a physiological pH. Assuming that about the first 20 residues of the sequence are lost as a signal peptide (see below), the net excess of negatively charged residues over the sum of the lysine, arginine and histidine residues is 12, consistent with the observed pI of 4.5 for the periplasmic enzyme (Scahill, 1981).

(ii) **Prediction of the cleavage site of signal peptide.** The *Rps. capsulata* enzyme is located in the periplasm, from which it can be released by cold osmotic shock (Scahill, 1981), and so the sequence would be expected to contain an *N*-terminal signal peptide to mediate the translocation from the cytoplasm. The signal peptides for the periplasmic cytochromes c_2 of *Rps. capsulata* (Daldal *et al.*, 1986) and *Rps. sphaeroides* (Donohue *et al.*, 1986) have been characterized. Each contain 21 residues, with one or two basic residues near the *N*-terminus, followed by a hydrophobic segment consisting of 15–19 uncharged residues. A similar *N*-terminal sequence is seen in the *Rps. capsulata* β -lactamase, and we predict that the *N*-terminus of the mature protein will be at about residue 25.

(iii) **Nucleic-acid-sequence relationships.** It was observed in the gene-hybridization experiment (Fig. 4) that the rhodospseudomonad β -lactamase gene did not hybridize to the TEM-type gene on pBR322. This is expected from the 'wobble' nature of the genetic code, which allows flexibility in the third base of a triplet so that it is often dependent on the G+C content of the organism (Dayhoff, 1972). The nucleic acid sequences of the gene in these investigations, as well as those of three other class A β -lactamases (the pBR322 *amp*, *Ps. aeruginosa* RMS 149 and *B. licheniformis* β -lactamase genes), were aligned according to the protein-sequence alignment in Fig. 5 and examined for nucleotide polymorphism. The principles used were the same as Kreitman's (1983) for alcohol dehydrogenase genes. The

sequences before the first invariant glutamic acid residue were not included, so as to avoid the effect of the different signal peptides.

As can be observed in Fig. 6, there has been considerable divergence in the class A β -lactamase genes, with the result that, in 38 of the 43 invariant amino acids, all possible variations in the third position of the codons are present. On the other hand, there are hardly any insertions or deletions, so that most of the changes have occurred by nucleotide substitution. Since all the proteins have β -lactamase function, these substitutions may be considered neutral.

(iv) **Similarity matrix of class A β -lactamase.** A pairwise comparison of the characterized class A protein sequences was carried out, and a similarity-matrix Table was drawn (Table 2). All amino acid residue matches were scored as '1' and mismatches were scored as 'zero'. The percentage similarity was then calculated from the total number of matches divided by the number of residues in the longer of the pair.

The results shown in Table 2 should not be interpreted in terms of a phylogeny for class A β -lactamase genes. The 60% similarity of the two enterobacterial enzymes from *E. coli* (TEM-type) and *Klebsiella pneumoniae* emphasizes that no fundamental distinction should be drawn between chromosomal and extrachromosomal genes, whereas the two bacillary sequences are not particularly similar to each other. Otherwise no pattern is apparent, even between Gram-positive and Gram-negative bacteria. There are, however, no instances of anomalous similarities that might suggest recent lateral gene transfer between disparate organisms. The results are similar to those observed in other cases where extensive sequence information is available for proteins of similar sequence from diverse organisms, such as for cytochromes *c* (Ambler *et al.*, 1987). One interpretation of such results (Meyer *et al.*, 1986) is that they may reflect the limits of divergence for a macromolecule that has to perform a specific function, with a value of percentage similarity for a particular structure and function that represents the equilibrium between divergence and convergence.

DISCUSSION

The *Rps. capsulata* sp108 β -lactamase gene was located to a 2.37 kb *SalI*/*Bam*HI DNA fragment of the 5.8 kb

Table 2. Similarity-matrix table of class A β -lactamase protein sequence

This is drawn from the alignment in Fig. 5. The *Kleb. pneumoniae* LEN sequence of Arakawa *et al.* (1986) has been included. Values are the number of identical residues expressed as percentages of total number of amino acids in the longer of the pair compared.

<i>Staphylococcus aureus</i> PC1	1	—							
<i>B. cereus</i> 569/H	2	34	—						
<i>B. licheniformis</i> 749/C	3	37	48	—					
<i>E. coli</i> TEM	4	29	30	30	—				
<i>Ps. aeruginosa</i> RMS 149	5	27	34	30	40	—			
<i>Rps. capsulata</i> sp108	6	21	26	26	36	36	—		
<i>Kleb. pneumoniae</i> LEN	7	23	30	30	60	41	37	—	
		1	2	3	4	5	6	7	

*Bam*HI insert in pSS20. Its nucleic acid sequence was determined, and the amino acid sequence of a β -lactamase, clearly recognized as a class A enzyme (Ambler classification), was deduced. This class of β -lactamase is commonly found in Gram-negative bacterial plasmids (Ambler & Scott, 1978), but hybridization studies and its proximity to *ampR* which is on chromosomes in some other Gram-negative bacteria, strongly suggest a chromosomal location for the *Rps. capsulata* sp108 β -lactamase gene. β -Lactamase genes are often organized as part of a transposable element (Richmond *et al.*, 1980) which can move between bacterial replicons independently of the cell's generalized recombination systems. Thus they can exist as part of a chromosome, plasmid or phage DNA. In TnA, the β -lactamase gene ends 110 bp upstream from the start of the inverted repeats required for transposition (Heffron *et al.*, 1979). The DNA sequence here only extends 119 bp downstream of the β -lactamase stop codon. This means that there was not enough sequence information to check for TnA inverted repeat sequences.

Its 68.5% G + C content, characteristic of rhodopseudomonad species (cf. *E. coli*, 48–52% G + C) and an amino acid sequence similarity with the TEM-type enzyme of only 36% would seem to indicate that this is an authentic rhodopseudomonad gene. A homologous β -lactamase gene which is chromosomal occurs universally in *Klebsiella pneumoniae* (Arakawa *et al.*, 1986). Those authors have postulated that as it is highly similar to the TEM-type enzyme, it might represent the ancestral gene of the common enterobacterial β -lactamase. The report herein of another inducible chromosomal class A β -lactamase in a photosynthetic bacterium with a 36% similarity might suggest that the class A β -lactamase gene is widely distributed and hence may be more central to the bacterium than has been heretofore envisaged. Interestingly, analysis of the DNA sequence upstream from the rhodopseudomonad β -lactamase gene revealed the presence of an *ampR* gene. This gene has been reported in several Gram-negative bacteria and encodes a regulatory protein which both represses *ampC* expression and modulates the induction of the universal class C enzyme by β -lactam antibiotics (Nicolas *et al.*, 1986). Though the β -lactamase involved here is a class A enzyme, the arrangement of the regulatory gene relative to the *amp* gene is similar, i.e. the *ampR* gene is located in the opposite strand, upstream to a divergently transcribed β -lactamase gene, both promoters overlapping in the intervening sequences. We have not mapped the rhodopseudomonad transcript and have not as such identified the relevant promoter(s). The similarity in the regulatory proteins and gene organization vis-à-vis the β -lactamase gene would suggest that the *ampR* product here may be functionally similar to that mediating *ampC*-protein induction in other Gram-negative bacteria.

In *Ent. cloacae* strains P99, MHN1, Q908R and in *Citrobacter freundii* where *ampR* has been characterized, it occurs downstream of the fumarate reductase (*frd*) operon. The putative amino acid sequence translated from the DNA upstream from the *ampR* gene in *Rps. capsulata* did not show any similarity to any of the genes of the *frd* operon. Thus the *ampR*/ β -lactamase complex in the rhodopseudomonad probably occurs in a location unrelated to that occupied by the *ampR*/*ampC* operon.

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