Chromosomal Loci Associated with Antibiotic Hypersensitivity in Pulmonary Isolates of *Pseudomonas aeruginosa*

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492a and 492c were two strains of *Pseudomonas aeruginosa* isolated from the sputum of a patient with cystic fibrosis. The strains were closely related but expressed different antibiograms. 492c was hypersensitive (10-100 times more sensitive than 492a) to the β -lactam antibiotics carbenicillin, methicillin, flucloxacillin, mecillinam and cefuroxime and the non- β -lactam, nalidixic acid. 492c also showed enhanced sensitivity (4-8 times more sensitive than 492a) to chloramphenicol, trimethoprim and novobiocin. 492a and PAO8 expressed similar levels of antibiotic resistance, except for trimethoprim, to which 492a was five times more sensitive than PAO8. Two genes associated with antibiotic hypersensitivity were mapped in the 30 min region of the chromosome, by means of R68.45-mediated plate matings between a Leu⁻ mutant of 492c and PAO8, followed by transductional analysis using phage F116L. The first of these genes, *blsA1*, was closely linked to *nalB*, and in a PAO background, was associated with hypersensitivity to the β -lactams and a moderate increase in sensitivity to chloramphenicol, trimethoprim, nalidixic acid and novobiocin. A further increase in sensitivity to the latter three antibiotics was associated with the second gene, *tpsA1*, which mapped between *ser-3* and *hisV*. This gene could also be transferred to PAO from 492a, thus 492c could have arisen from 492a *in vivo* following a single chromosomal mutation at the *blsA* locus. Isolation of a *blsA* mutant of PAO969 provided further evidence for this theory.

INTRODUCTION

One of the characteristics most commonly associated with *Pseudomonas aeruginosa* is an intrinsic resistance to antimicrobial agents, in particular, the β -lactam antibiotics. Indeed, this property is generally held responsible for the emergence of *P. aeruginosa* in the last three decades as a clinically important opportunist pathogen.

The development of carbenicillin, the first semi-synthetic penicillin with significant anti-pseudomonas activity, was a significant advance in chemotherapy. However, the minimal inhibitory concentration (MIC) of carbenicillin for *P. aeruginosa* is generally 25-50 $\mu\text{g ml}^{-1}$ (Knudsen *et al.*, 1967), which is beyond the levels attainable in soft tissues, e.g. the lung (Marks *et al.*, 1971).

May & Ingold (1972) reported that strains of *P. aeruginosa* isolated from sputum were often considerably more sensitive to carbenicillin than isolates from other sources. In a study involving 111 sputum isolates from patients with cystic fibrosis (CF), chronic bronchitis and bronchiectasis, they found that 35% of strains were sensitive to 6 μg carbenicillin ml^{-1} and some had MICs as low as 0.7 $\mu\text{g ml}^{-1}$.

Abbreviations: CEF, cefuroxime; CF, cystic fibrosis; DSTA, Diagnostic Sensitivity Test Agar; NA, nutrient agar; NB, nutrient broth; TP, trimethoprim.

Berche *et al.* (1979) reported a study on antibiotic susceptibilities of mucoid and non-mucoid strains of *P. aeruginosa*. They compared 47 mucoid with 71 non-mucoid isolates on the basis of sensitivities to 18 antibiotics and concluded that both groups could be divided into two distinct classes on this basis. Class A contained strains significantly more resistant to antibiotics such as the aminoglycosides and tetracycline, than those strains allocated to Class B.

While studying the association of *P. aeruginosa* with chronic respiratory infection in CF patients, we have also reported the isolation of strains unusually sensitive (hypersensitive) to a range of antibiotics including the β -lactams carbenicillin, azlocillin, methicillin and also to trimethoprim (Irvin *et al.*, 1981). The carbenicillin MICs of these strains were $<1 \mu\text{g ml}^{-1}$ and thus comparable with those reported by May & Ingold (1972). In addition, we reported that hypersensitive and 'normal' isolates could be obtained from the same sputum specimen and that these strains were invariably of the same pyocin type, indicating a close relationship.

Recently, there has been an increasing interest in the genetic basis of virulence in *P. aeruginosa* and various potential virulence factors have been mapped using the genetically characterized strain, PAO. For example, several chromosomal genes involved in exotoxin A production have been mapped following isolation of toxin-deficient PAO mutants, and the use of classical genetic techniques (Gray & Vasil, 1981*a*; Hanne *et al.*, 1983). Similarly, a chromosomal gene controlling phospholipase C production has been identified (Gray & Vasil, 1981*b*). Both of these potential virulence factors are normally expressed in PAO, but others may be expressed only following a mutation, for example, alginate production leading to a mucoid phenotype which is so common in *P. aeruginosa* isolated from the sputa of CF patients. Mucoid mutants of PAO have been isolated *in vitro* and two chromosomal loci involved in the control of alginate synthesis have been mapped (Govan & Fyfe, 1978; Fyfe & Govan, 1980; J. A. M. Fyfe & J. R. W. Govan, unpublished).

A third approach to the genetic analysis of virulence involves the transfer of the relevant gene(s) from a clinical isolate into a PAO background. This approach is applicable to both chromosomally-encoded and plasmid-encoded characteristics, and has been used in the genetic analysis of extracellular protease production (Wretling *et al.*, 1983) and transposable antibiotic resistance (Sinclair & Holloway, 1982).

In the course of studying the intrinsic antibiotic resistance of *P. aeruginosa* strain PAO, various loci associated with this characteristic have been mapped on the chromosome (Mills & Holloway, 1976; Okii *et al.*, 1983; Matsumoto & Terawaki, 1982). Functional aspects of the *P. aeruginosa* outer membrane have been examined using an antibiotic 'supersusceptible' mutant of strain 799 (Zimmerman, 1980; Angus *et al.*, 1982; Darveau & Hancock, 1983). However, genetic analysis of this strain proved unsuccessful (Angus *et al.*, 1982).

The genetic basis of antibiotic hypersensitivity as expressed in clinical isolates of *P. aeruginosa* has not, to our knowledge, been reported. May & Ingold (1972) postulated that the normal resistance to carbenicillin might be plasmid-encoded, and that this plasmid may sometimes be lost during growth in the respiratory tract. However, no evidence was presented to support this hypothesis. The present paper describes the genetic mapping of antibiotic hypersensitivity in the clinical isolate 492c (Irvin *et al.*, 1981) by means of inter-strain crosses with suitably marked PAO recipients, followed by transductional analysis using phage F116L (Krishnapillai, 1971). A preliminary report of this work has been presented (Fyfe & Govan, 1983).

METHODS

Bacteria and bacteriophages. The bacterial strains used in this study are shown in Table 1. Donor strains carrying the plasmid R68.45 were constructed according to Haas & Holloway (1976). Phage F116L (Krishnapillai, 1971) was used for transduction and F116c for strain construction.

Media and cultural conditions. Nutrient broth (NB), nutrient agar (NA), and minimal agar have been described previously (Fyfe & Govan, 1980). Amino acid supplements were added at a concentration of 1 mM. The antibiotics used were carbenicillin (Pyopen; Beecham), methicillin (Celbenin; Beecham), benzyl penicillin (Crystapen; Glaxo), ampicillin (Penbritin; Beecham), flucloxacillin (Floxapen; Beecham), mecillinam (Leo Pharmaceuticals), cefuroxime (Zinacef; Glaxo), cefoxitin (Mefoxin; MSD), tetracycline (Glaxo), trimethoprim lactate (Wellcome), nalidixic acid (Winthrop), novobiocin (Sigma), rifampicin (Roche), streptomycin (Glaxo), gentamicin (Roussel),

Table 1. Strains of *P. aeruginosa* used in this study

Strain	Genotype/Description*	Reference
PAO1	Prototroph, <i>chl</i> -2	Holloway (1969)
PAO2	<i>ser</i> -3	Isaac & Holloway (1968)
PAO4	<i>arg</i> -47 <i>pyr</i> B52	B. W. Holloway collection
PAO8	<i>met</i> -28 <i>ilv</i> -202 <i>str</i> -1	Isaac & Holloway (1968)
PAO222	<i>met</i> -28 <i>trp</i> -6 <i>lys</i> -12 <i>his</i> -4 <i>ilv</i> -226 <i>pro</i> -82	Haas & Holloway (1976)
PAO969	<i>pro</i> C130	Rella & Haas (1982)
PAO6002	<i>met</i> -9011 <i>ami</i> E200 <i>nal</i> B4	Rella & Haas (1982)
PAO6006	<i>pro</i> C130 <i>nal</i> B9	Rella & Haas (1982)
PAO6511	<i>pro</i> C130 <i>bls</i> A2	This paper
PAO6524	<i>pro</i> C130 <i>nal</i> B4 derivative constructed using F116c	This paper
PAO6526	Pro ⁺ transductant of PAO6511 constructed using F116c	This paper
GMA037	<i>his</i> V5037	Mee & Lee (1967)
492a	Clinical isolate, mucoid, prototrophic, <i>tps</i> A	Irvin <i>et al.</i> (1981)
492a Leu ⁻	Leucine auxotroph isolated from 492a following EMS mutagenesis	This paper
492c	Clinical isolate, mucoid, prototrophic, <i>bls</i> A1 <i>tps</i> A1	Irvin <i>et al.</i> (1981)
492c Leu ⁻	Leucine auxotroph isolated from 492c following EMS mutagenesis	This paper
PAJ1	Prototrophic, <i>tps</i> A1 <i>bls</i> A1 recombinant from cross 492c Leu ⁻ R68.45 × PAO8	This paper
PAJ2	Prototrophic, <i>bls</i> A1 recombinant from cross 492c Leu ⁻ R68.45 × PAO8	This paper
PAJ3	Prototrophic, <i>tps</i> A1 recombinant from cross 492c Leu ⁻ R68.45 × PAO8	This paper
PAJ5	<i>met</i> -28 <i>tps</i> A1, recombinant from cross 492c Leu ⁻ R68.45 × PAO8	This paper
PAJ6	<i>ilv</i> -202 <i>tps</i> A1, transductant of PAJ5 using F116c grown on PAO8 <i>met</i> ⁺	This paper

* Genotype symbols are the same as those used for *E. coli*; *bls*A designates sensitivity to β -lactam antibiotics, *tps*A sensitivity to trimethoprim, *ami* acetamide utilization and *str* resistance to streptomycin.

tobramycin (Lilley), and kanamycin (Bristol Laboratories). All antibiotics were incorporated in Diagnostic Sensitivity Test Agar (DSTA; Oxoid) at the appropriate concentration.

Broth cultures were grown in McCartney bottles on an orbital incubator (Gallenkamp) at 140 r.p.m., and all cultures were incubated at 37 °C, unless stated otherwise.

Isolation of mutants. Auxotrophic mutants were isolated after EMS mutagenesis (Watson & Holloway, 1976) and carbenicillin enrichment (Watson & Holloway, 1978).

Carbenicillin-hypersensitive mutants were isolated following EMS mutagenesis and screening of colonies by replica plating onto DSTA plates containing 5 μ g carbenicillin ml⁻¹.

Antibiotic sensitivity testing. Exponential phase NB cultures were diluted in physiological saline to a concentration of 10⁵ organisms ml⁻¹. A multiple inoculator (Mast) was used to dispense samples (containing 10²–10³ cells) onto DSTA plates appropriately supplemented with the antibiotics. The MIC was read as the lowest concentration of antibiotic that caused complete growth inhibition after 18 h incubation at 37 °C.

Plate matings. The procedure used was that described by Stanisich & Holloway (1972). Prior to inter-strain matings, NB cultures of the recipient strains were incubated for 18 h at 43 °C (Rolfe & Holloway, 1966).

Transductions. These were performed according to the method of Krishnapillai (1971).

RESULTS

Antibiotic sensitivities

It has previously been reported that 492a and 492c differ in their sensitivities to carbenicillin, azlocillin, piperacillin, ticarcillin, methicillin, tetracycline and trimethoprim (Irvin *et al.*, 1981).

We determined the MICs of an extended range of antibiotics for 492a and 492c, and the results are shown in Table 2. Strain PAO8 was also included to confirm that the MICs obtained for this strain were comparable with 492a and not the hypersensitive 492c. However, 492a was five times more sensitive to trimethoprim than PAO8. Table 2 shows that 492c is generally hypersensitive to the β -lactam antibiotics with the following exceptions: benzyl penicillin,

Fig. 1. Genetic map of *P. aeruginosa* showing markers relevant to this study. Based on Holloway & Crockett (1982).

Table 2. Sensitivities of *P. aeruginosa* strains 492a, 492c and PAO8 to antibiotics

Antibiotic	Strain ...	MIC ($\mu\text{g ml}^{-1}$)		
		492a	492c	PAO8
Carbenicillin		80	0.6	40
Methicillin		> 500	10	500
Benzyl penicillin		500	500	> 1000
Ampicillin		100	100	200
Flucloxacillin		> 500	50	> 500
Mecillinam		500	10	500
Cefuroxime		> 500	10	400
Cefoxitin		400	400	500
Cephaloridine		500	500	> 1000
Chloramphenicol		40	10	60
Tetracycline		10	6	10
Trimethoprim		40	5	200
Nalidixic acid		60	2	80
Novobiocin		> 500	100	500
Rifampicin		40	40	20
Streptomycin		40	40	> 500*
Gentamicin		0.6	0.6	0.6
Tobramycin		0.4	0.4	0.2
Kanamycin		60	60	60

* PAO8 is resistant to streptomycin due to the *str-1* locus.

ampicillin, cefoxitin and cephaloridine. Increased sensitivity to trimethoprim was confirmed for 492c, and also noted for nalidixic acid and novobiocin. No differences between 492a and 492c were observed for rifampicin, streptomycin, gentamicin, tobramycin or kanamycin.

Transfer of antibiotic hypersensitivity from 492c to PAO

Figure 1 is a chromosome map of *P. aeruginosa* PAO showing the locations of markers relevant to this study. A donor derivative of 492c was prepared by isolating a *Leu*⁻ mutant, and

Table 3. Results of recombinant analysis following a plate mating between 492c *Leu*⁻ (R68.45) and PAO8

Selected marker	Percentage co-inheritance of unselected character			
	CEF ^s *	TP ^s †	Met ⁺	Ilv ⁺
<i>met-28</i> ⁺	80	82	—	77
<i>ilv-202</i>	75	88	87	—

* CEF^s designates sensitivity to 100 µg cefuroxime ml⁻¹.

† TP^s designates sensitivity to 100 µg trimethoprim ml⁻¹.

transferring R68.45 into this strain. Plate matings were performed between 492c *Leu*⁻ (R68.45) and the multiple-marker recipient PAO222. Selection was made for each of the auxotrophic markers and 100 recombinants for each marker were scored for co-inheritance of cefuroxime sensitivity (on DSTA plus 100 µg cefuroxime ml⁻¹). Increased sensitivity to cefuroxime rather than carbenicillin was chosen initially to avoid any problems associated with the inheritance of R68.45 by some of the recombinants (R68.45 codes for a type IIIa β -lactamase which is active against carbenicillin but not cefuroxime). Recombinant analysis revealed that 56% of the *met-28*⁺ colonies were sensitive to 100 µg cefuroxime ml⁻¹ and likewise 20% of the *trp-6*⁺ colonies. Less than 1% of the recombinants for the other markers, i.e. *ilv-226*, *his-4*, *lys-12* and *pro-82* had co-inherited this characteristic.

In order to map the locus associated with increased cefuroxime sensitivity more precisely, a plate mating was performed between 492c *Leu*⁻ (R68.45) and PAO8 (*met-28 ilv-202*). Selection was made for both auxotrophic markers and recombinants scored for both cefuroxime and trimethoprim sensitivity (on DSTA plus 100 µg trimethoprim ml⁻¹). The results of this recombinant analysis are shown in Table 3, and indicate that cefuroxime sensitivity (CEF^s) and trimethoprim sensitivity (TP^s) do not always cosegregate. All CEF^s recombinants were TP^s, but a percentage of the TP^s colonies were CEF^r.

The MICs for representatives of each recombinant class, i.e. CEF^s TP^s, CEF^r TP^s and CEF^r TP^r, were determined using those antibiotics to which 492c is hypersensitive, i.e. carbenicillin, methicillin, flucloxacillin, mecillinam, cefuroxime, chloramphenicol, trimethoprim, nalidixic acid and novobiocin.

All CEF^r TP^r recombinants (three tested) were indistinguishable from PAO8 on the basis of MICs to these antibiotics. The CEF^s TP^s group (eight tested) could be subdivided on the basis of their trimethoprim, nalidixic acid and novobiocin MICs. Three recombinants were very sensitive to these antibiotics with trimethoprim and novobiocin MICs of 10 µg ml⁻¹, and a nalidixic acid MIC of 5 µg ml⁻¹. The other CEF^s TP^s recombinants were moderately sensitive with the following MICs: trimethoprim 60 µg ml⁻¹, novobiocin 50 µg ml⁻¹, nalidixic acid 20 µg ml⁻¹. All eight recombinants were equally sensitive to the β -lactam antibiotics and chloramphenicol, and with the exception of cefuroxime were as sensitive as 492c. The third class of recombinant, CEF^r TP^s (five tested), displayed moderate sensitivity to trimethoprim, nalidixic acid and novobiocin, with MICs of 60 µg ml⁻¹, 20 µg ml⁻¹ and 100 µg ml⁻¹, respectively.

From these results, we postulated that the antibiotic hypersensitive phenotype of 492c is determined by two separate genes, both linked to *met-28* and *ilv-202*, when transferred into PAO8. We propose to call these genes *blsA1*, coding for hypersensitivity to certain β -lactams and a moderate sensitivity to trimethoprim, nalidixic acid and novobiocin; and *tpsA1* coding for increased sensitivity to the latter three antibiotics but not the β -lactams. Table 4 shows the relevant MIC values for PAO8 and three typical recombinant strains PAJ1 (*blsA1 tpsA1*), PAJ2 (*blsA1*) and PAJ3 (*tpsA1*).

Transfer of *tpsA1* from 492a to PAO8

As shown in Table 2, the trimethoprim MIC for 492a is fivefold less than for PAO8. In addition, the increased sensitivity of 492c to trimethoprim, compared with 492a (eightfold), is of the same order as the MIC difference between PAJ1 and PAJ3 (sixfold) (see Table 4).

Fig. 2. Approximate locations of *blsA* and *tpsA* on the basis of results obtained from plate matings between 492c Leu⁻ (R68.45) and PAO8.

Table 4. Antibiotic sensitivities of PAO8 and typical recombinant strains PAJ1, PAJ2 and PAJ3

Antibiotic	Strain ...	MIC ($\mu\text{g ml}^{-1}$)			
		PAO8	PAJ1 (<i>blsA1 tpsA1</i>)	PAJ2 (<i>blsA1</i>)	PAJ3 (<i>tpsA</i>)
Carbenicillin		40	0.4	0.4	40
Methicillin		500	10	10	500
Flucloxacillin		> 500	50	50	> 500
Mecillinam		500	10	10	500
Cefuroxime		400	100	100	400
Chloramphenicol		40	20	20	40
Trimethoprim		200	10	60	60
Nalidixic acid		80	5	20	20
Novobiocin		500	10	50	10

On this basis, we proposed that *tpsA1* is present in both 492a and 492c, and that the antibiotic hypersensitivity of the latter strain can be explained by a mutation at the *blsA* locus, i.e. there is only a single gene difference between 492a and 492c accounting for antibiotic hypersensitivity.

Using the mapping procedure already described for 492c, a donor derivative of 492a [492a Leu⁻ (R68.45)] was plate mated with PAO8, and the *met*⁺ and *ilv*⁺ recombinants scored on DSTA containing either 100 μg trimethoprim ml^{-1} or 100 μg cefuroxime ml^{-1} . As expected, a proportion of recombinants failed to grow well on trimethoprim, though all grew well on cefuroxime.

Mapping of blsA and tpsA by transduction using phage F116L

From the plate mating results, it was possible to assign approximate chromosomal locations to *blsA1* and *tpsA1* relative to *ilv-202* and *met-28* (see Fig. 2). However, to map these loci more precisely with respect to other markers in the 30 min region, transductional analysis was performed.

In the case of *blsA1*, a preparation of F116L was grown on PAJ2 and used to transduce recipient strains carrying the auxotrophic markers *met-28* (PAO8), *ilv-202* (PAO8), *proC130* (PAO969) and *pyrB52* (PAO4). Selection was made for these markers and cotransduction of *blsA1* scored on DSTA plus 5 μg carbenicillin ml^{-1} .

In the case of *tpsA1*, transducing preparations of F116L could not be propagated on any recombinants containing this marker, as they did not appear to support vegetative growth. However, *tpsA1* strains retained the receptors for this phage as they were good recipients in F116L-mediated transductions, so in order to map this locus, an *ilv-202 tpsA1* derivative, PAJ6, was constructed (via the intermediate strain PAJ5) and used as recipient in transductions mediated by F116L propagated on either PAO1, PAO2 (*ser-3*) or GMA 037 (*hisV5037*). In each case selection was made for *ilv*⁺ transductants and these were scored for co-inheritance of *tpsA*⁺ and where appropriate, the unselected auxotrophic markers. Figure 3 shows the locations obtained for *blsA* and *tpsA*.

Isolation of a blsA mutant of PAO969

Working on the hypothesis that 492c could have arisen from 492a by a single step mutation at the *blsA* locus, we postulated that a similar *blsA* mutant could be isolated from a PAO strain.

Fig. 3. Mapping of *tpsA1* and *blsA1* by transduction. Values indicate percentage cotransduction using phage F116L (the mean of several experiments scoring at least 200 transductants). Arrowheads point to the unselected marker.

Table 5. Results of F116L-mediated transductions to determine the relationship between *nalB* and *blsA*

F116L grown on:	Recipient	Percentage cotransduction with <i>proC</i> **		
		<i>blsA2</i>	<i>nalB4</i>	Wild-type
PAO1 (<i>nalB4</i> ⁺ <i>blsA2</i> ⁺)	PAO6511 (<i>blsA2</i>)	—	—	33
	PAO6524 (<i>nalB4</i>)	—	—	43
PAO6002 (<i>nalB4</i>)	PAO6511 (<i>blsA2</i>)	—	27	<0.5
PAO6526 (<i>blsA2</i>)	PAO6524 (<i>nalB4</i>)	19	—	3

* In each case, selection was made for *proC*⁺ and 200 transductants were scored for the co-inheritance of *nalB4*^{+/−} and *blsA2*^{+/−} on DSTA plus 50 µg and 5 µg carbenicillin ml^{−1}, respectively.

PAO969 was mutagenized with EMS and the potential mutant colonies screened for hypersensitivity to carbenicillin by replica plating to DSTA plates containing 5 µg carbenicillin ml^{−1}. From approximately 5000 colonies screened, one failed to grow on medium containing carbenicillin. Fortunately, this mutant (PAO6511) exhibited a similar antibiogram to the *blsA1* recombinants, i.e. PAO6511 was highly sensitive to carbenicillin, moderately sensitive to cefuroxime, trimethoprim, nalidixic acid, novobiocin and chloramphenicol, but remained resistant to ceftiofloxacin, cephaloridine and benzyl penicillin. However, PAO6511 was more resistant to methicillin, mecillinam and flucloxacillin than a *blsA1* transductant of PAO969.

F116L transductions revealed 33% cotransduction between *proC* and the mutant locus. Furthermore, when F116L propagated on PAJ2 was used to transduce PAO6511, selecting for *proC*⁺, <0.5% of the transductants were able to grow on 5 µg carbenicillin ml^{−1}, indicating a very close linkage between *blsA1* and the PAO mutation, which, on this basis, we have designated *blsA2*.

Relationship between *blsA* and *nalB*

Rella & Haas (1982) have described a class of mutants (*nalB*) in strain PAO which are resistant to nalidixic acid, novobiocin, carbenicillin and various other β-lactams. We examined two *nalB* mutants PAO6002 (*nalB4*) and PAO6006 (*nalB9*) to determine whether they showed increased resistance to cefuroxime, trimethoprim, methicillin and chloramphenicol. Both strains showed increased resistance to all four antibiotics with the following relative MICs with respect to PAO969: cefuroxime, × 3; trimethoprim, × 6; methicillin, × 5; chloramphenicol, × 10.

nalB has been mapped at 32 min on the PAO chromosome, distal to *pyrB* using G101 transduction (Rella & Haas, 1982), so we determined the location of *blsA2* with respect to this locus, using strains PAO6511 and PAO6002. In order to perform reciprocal transductions, a *proC* derivative of PAO6002 (PAO6524) and a *proC*⁺ derivative of PAO6511 (PAO6526) were

constructed using F116c. Reciprocal transductions using F116L were performed between the *blsA* and *nalB* strains, in each case selecting for Pro⁺ transductants and scoring for *blsA* and *nalB* on DSTA plates containing 5 µg and 50 µg carbenicillin ml⁻¹. The results obtained are shown in Table 5, and suggest that *blsA* and *nalB* are separate, but closely linked loci, the gene order being *proC-nalB-blsA*.

DISCUSSION

Previous genetic studies on the intrinsic resistance of *P. aeruginosa* strain PAO have identified various chromosomal loci associated with this characteristic. Thus we tested the hypothesis that the loss of intrinsic resistance in 492c was due to a chromosomal mutation, rather than loss of a plasmid as suggested by May & Ingold (1972). Inter-strain crosses between 492c Leu⁻ (R68.45) and PAO8 identified two distinct loci, in the 30 min region of the chromosome, associated with antibiotic sensitivity. The first of these, *blsA*, is associated with sensitivity to the β-lactams carbenicillin, methicillin, flucloxacillin, mecillinam and cefuroxime, and also the non-β-lactams chloramphenicol, trimethoprim, nalidixic acid and novobiocin and cannot be transferred to PAO from 492a.

The isolation of a *blsA* derivative of PAO following EMS mutagenesis provides further evidence that 492c could have arisen from 492a *in vivo* following a single-step mutation which was then selected out in the lung environment. This mutation-selection mechanism is also responsible for the emergence of mucoid strains of *P. aeruginosa* during the course of chronic pulmonary infections in CF patients. All strains of *P. aeruginosa* have the necessary structural genes to synthesize alginate, but these are normally subject to repression. Mutation in a regulator gene close to the major FP2 origin results in derepression of alginate synthesis giving rise to a mucoid strain (Govan & Fyfe, 1978; Fyfe & Govan, 1980; J. A. M. Fyfe & J. R. W. Govan, unpublished).

Three major questions remain to be answered regarding antibiotic hypersensitivity associated with respiratory isolates. Firstly, whether all such isolates have the same mutation as 492c or whether they form a genetically heterogeneous group. We have examined several pairs of strains (i.e. hypersensitive and 'normal' from the same sputum specimen) isolated from different patients on the basis of antibiotic sensitivities and in general, the antibiograms have been similar to 492a and 492c (results not shown). Genetic analysis of one other hypersensitive strain gave identical results to those obtained with 492c, even though this strain was isolated from a different patient and was of a different pyocin type. However, further work is required to determine whether the *blsA* locus is the only one involved in clinically associated antibiotic hypersensitivity.

Secondly, we would like to know the structural basis of this characteristic. We have previously reported that 492c has two extra outer membrane proteins when compared with 492a (Irvin *et al.*, 1981), but further studies have failed to confirm that these are associated with antibiotic sensitivity.

Several studies on the structural basis of antibiotic hypersensitivity in *P. aeruginosa* have been reported (Noguchi *et al.*, 1980; Curtis *et al.*, 1981; Angus *et al.*, 1982; Zimmerman, 1980; Kropinski *et al.*, 1982; Darveau *et al.*, 1983) and these have suggested that increased permeability of the cell envelope is responsible for this characteristic. In the case of 799/61, a 'supersusceptible' mutant of strain 799 (Zimmerman, 1979), alterations in the LPS composition have been detected (Kropinski *et al.*, 1982; Darveau *et al.*, 1983). Similar mutants (*envA*) have been reported in *Escherichia coli* (Grundstrom *et al.*, 1980; Boman *et al.*, 1974).

The close linkage of *blsA* and *nalB* on the *P. aeruginosa* chromosome is interesting in view of the fact that *nalB* mutants are more resistant to those antibiotics to which *blsA* strains are hypersensitive, and it is tempting to speculate that these two loci form part of a gene cluster controlling outer membrane permeability in this organism.

Finally, the clinical and biological significance of antibiotic hypersensitivity remains to be elucidated. Superficially it appears paradoxical that hypersensitive strains arise in patients undergoing aggressive antibiotic therapy, often with β-lactams. The lung, however, is a

notoriously difficult site in which to achieve therapeutic levels of anti-pseudomonas antibiotics, and in this environment the vulnerability of hypersensitive strains may be more apparent than real.

Mutations to increased antibiotic sensitivity are also encountered amongst naturally occurring gonococci (Eisenstein & Sparling, 1978; Lysko & Morse, 1981) and it has been suggested that mucosal surface pathogens may find it advantageous to be flexible with respect to outer membrane structure and that increased permeability may aid colonization in this environment (Lysko & Morse, 1981).

The emergence of antibiotic-hypersensitive strains of *P. aeruginosa* during the course of chronic pulmonary infections, regardless of the selective pressure, provides yet another example of this organism's ability to adapt to a changing environment.

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