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Physical mapping of 32 genetic markers on the *Pseudomonas aeruginosa* PAO1 chromosome

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The *Pseudomonas aeruginosa* chromosome was fractionated with the enzymes *Spe*I and *Dpn*I, and genomic fragments were separated by PFGE and used for mapping a collection of 40 genes. This permitted the localization of 8 genes previously mapped and of 32 genes which had not been mapped. We showed that a careful search of databases and identification of sequences that were homologous to known genes could be used to design and synthesize DNA probes for the mapping of *P. aeruginosa* homologues by Southern hybridization with genomic fragments, resulting in definition of the locations of the *aro-2, dapB, envA, mexA, groEL, oprH, oprM, oprP, ponA, rpoB* and *rpoH* genetic markers. In addition, a combination of distinct DNA sources were utilized as radioactively labelled probes, including specific restriction fragments of the cloned genes (*glpD, opdE, oprH, oprO, oprP, phoS*), DNA fragments prepared by PCR, and single-stranded DNA prepared from phagemid libraries that had been randomly sequenced. We used a PCR approach to clone fragments of the putative *yhhF, sucC, sucD, cypH, pbpB, murE, pbpC, soxR, ftsA, ftsZ* and *envA* genes. Random sequencing of *P. aeruginosa* DNA from phagemid libraries and database searching permitted the cloning of sequences from the *acoA, catR, hemD, pheS, proS, oprD, pyo* and *rpsB* gene homologues. The described genomic methods permit the rapid mapping of the *P. aeruginosa* genome without linkage analysis.

Keywords: *Pseudomonas aeruginosa* chromosome, physical mapping, genetic markers

INTRODUCTION

The current genetic map of the *Pseudomonas aeruginosa* chromosome lists the location of more than 350 marker loci (Ratnaningsih *et al.*, 1990; Römling *et al.*, 1992; Holloway *et al.*, 1994). In addition, two physical maps of the 5·94±0·02 Mb large circular 65% G+C-rich chromosome have been constructed by either bottom-up or top-down mapping techniques (Römling *et al.*, 1989; Römling & Tümmler, 1991). The complete macrorestriction map of the 5·94 Mb genome was constructed by using 38 *Spe*I and 15 *Dpn*I sites, yielding a mean resolution of 110 kb including similar distances on the physical and genetic maps. The zero point of the *P. aeruginosa* strain PAO1 map has been relocated to the chromosomal origin of replication (Römling *et al.*, 1992). A combined physical and genetic map has been constructed by PFGE and Southern-type gel hybridization which identified 40 cosmid clones carrying known chromosomal markers, by complementation of *P. aeruginosa* auxotrophic mutants (Ratnaningsih *et al.*, 1990).

A modest number of virulence and metabolic genes have been precisely located on the combined physical and genetic map, whereas a computer search of available databases such as GenBank revealed more than 450 *Pseudomonas* 'gene' sequences; less than 40% of these have been localized on the chromosome map. Thus the potential for using synthetic probes for mapping is considerable. In addition, probes can also be rapidly prepared by PCR and positioned by hybridization.

Abbreviations: PBP, penicillin-binding protein; STS, sequence tagged sites.

In this paper, we report a survey of the *P. aeruginosa* genome in a relatively random manner and the analysis of sequences that are representative of distinct portions of the genome. The data obtained were compared to sequences available in the databases. A collection of eight sequences, referred to as sequence tagged sites (STS), were also localized on the *P. aeruginosa* physical map. The use of a combination of genomic methods has permitted extension of the number of mapped genes by 32 loci. We report the mapping and cloning of several genes encoding cell-wall-synthesizing enzymes, control of cell division, penicillin-binding proteins (PBPs) and outer-membrane proteins, and genes implicated in antibiotic resistance and general metabolism. We also confirm that genes can be mapped rapidly and to specific regions of the *P. aeruginosa* PAO genome by using synthetic oligonucleotide and single-stranded DNA probes obtained by random sequencing.

METHODS

Bacterial strains, plasmids and phages. The bacterial strain *P. aeruginosa* PAO1293, the prototype strain for preparation of genomic DNA for PFGE, was a derivative of PAO1 constructed from PAO2 using the transducing phage E79tv-2 and carried a mutation for chloramphenicol resistance (Ratnaningsih *et al.*, 1990). *Escherichia coli* JM101 was conserved on minimal media without proline. Bacterial cells were routinely grown on tryptic soy agar (Difco) containing appropriate levels of antibiotics (100 μg ampicillin ml^{-1}; 300 μg chloramphenicol ml^{-1}). Plasmids pTZ18R and pTZ18U were described previously (Vieira & Messing, 1987). For the pTZ18 phagemids, production of single-stranded DNA with the helper phage M13K07 was as described by Vieira & Messing (1987).

Preparation of DNA and related techniques. Plasmids were prepared by the cleared lysate method and purified by caesium chloride/ethidium bromide gradient ultracentrifugation. Plasmid DNA was digested with restriction endonucleases and analysed by agarose gel (0·7–1·5 %, w/v) electrophoresis using conditions recommended by the manufacturer (Pharmacia LKB Biotechnology). Subcloning of DNA fragments, T4 ligase conditions and transformation procedures are standard methods previously described by Sambrook *et al.* (1989).

Cloning of the *yhhF, sucC, cypH, pbpB/murE, pbpC/soxR* and *ftsA/ftsZ/envA* genes. In an attempt to clone the genes encoding the PBPs of *P. aeruginosa*, primers were designed as complementary to the SXXK and KTG boxes conserved in similar proteins from other bacterial species (Spratt & Cromie, 1988; Joris *et al.*, 1991). The nucleotide sequences synthesized as primers were 5′-TTTGAATTCGG(C)CA(T)C(G)C(G)G(AC)-C(AT)G(C)G(A)C(T)G(C)AAGCC-3′ [from the amino acid sequence of G(A)ST(ANL)V(IAM)KP] and 5′-AAAGAAT-TCG(CT)T(C)T(G)C(G)GT(C)C(G)GTGCCG(C)G(C)T(A)-CTT-3′ [from the amino acid sequence of KT(S)GT-T(A)N(QRK)], with flanking *Eco*RI recognition sequences, where the letters in parentheses represent alternative nucleotides and amino acids that were included in the primers and encoded by the primers, respectively, at given positions in the sequence corresponding to the preceding nucleotide or amino acid. To clone *ftsZ*, specific PCR primers were based on segments of amino acid identity between the *Bacillus subtilis*, *Rhizobium meliloti* and *E. coli* FtsZ proteins (Margolin *et al.*, 1991), adjusted to the codon usage of *P. aeruginosa* (West & Iglewski, 1988). The actual primers used were 5′-CCCAAGCGCTGAAGAAC-3′ and 5′-ATCACCACCGGCATGGG-3′. These amplified a 220 bp product in *P. aeruginosa* but not in *E. coli*.

PCR amplification. Chromosomal DNA was obtained by standard procedures and was amplified by PCR using an Ericomp thermal cycler with the following parameters. The reaction mixture consisted of 5 % (v/v) formamide/10 % (v/v) glycerol/15 mM Mg^{2+} and the reaction took place under the following conditions: the first five cycles involved temperature cycles of 94 °C for 15 s, 37 °C for 30 s and 72 °C for 90 s, whereas the primer annealing temperature was raised from 37 °C to 55 °C for the remaining 25 cycles. Products of amplification were identified on agarose gels (Sambrook *et al.*, 1989) and, after excision, cloned into plasmid pUC18.

Nucleotide sequence analysis and DNA synthesis. Some sequencing was done by the dideoxynucleotide polymerase chain-termination procedure with the T7 polymerase sequencing kit (Pharmacia) and [^{35}S]dATPαS (Amersham). In addition to the 17-mer universal primer (Pharmacia), a series of 17-mers and the collection of oligomers described in Table 2 were synthesized and used as probes or primers to completely sequence both DNA strands. Oligonucleotides used as probes were synthesized by the phosphoramidite method on a Beckman Oligo1000 synthesizer. The product was cleaved from the solid support, concentrated, deprotected and purified on polyacrylamide sequencing gels as suggested by the manufacturer. The PCR insert DNAs cloned into pTZ18U were sequenced using an ABI model 370A automated DNA sequencer with fluorescent dye terminator methodologies supplied by the manufacturer (Applied Biosystems). Sequenced genes were identified by translating all six possible reading frames and using the Geninfo(R) Blast Network service, which utilizes the basic local alignment search methodology of Robison *et al.* (1994).

Physical mapping of genes. *P. aeruginosa* genomic DNA was prepared in agarose blocks as suggested by Birren & Lai (1993) and fractionated with the enzymes *Dpn*I and *Spe*I in single and double digests. All gels were 1 % (w/v) agarose (SeaKem LE) and were electrophoresed at 6 V cm^{-1} for 20 h at 14 °C with a reorientation angle of 120 ° using the CHEF Gene Mapper (Bio-Rad) or the Hexafield apparatus (Pharmacia). Switch times were constant for a typical gel but separations were done at 15 s, 20 s and 25 s to shift the windows of resolution such that every *Spe*I and *Dpn*I genomic fragment, and double digests, could be separated. DNA fragments separated in agarose gels were transferred to nylon membranes by the Southern procedure (Sambrook *et al.*, 1989). These were then hybridized with probes prepared by various means, such as insert fragments from plasmids containing the cloned genes indicated in parentheses (Table 1), with PCR products, with oligo probes and with single-stranded DNA labelled with ^{32}P. Some experiments employed digoxigenin-labelled probes and PFGE as described by Lightfoot & Lam (1993).

RESULTS AND DISCUSSION

Cloning of the *yhhF, sucC, cypH, pbpB/murE, pbpC/soxR* and *ftsA/ftsZ/envA* genes

PCR amplification of the *P. aeruginosa* chromosome, using primers designed to amplify the genes for PBPs, led to five separate DNA sequences. Only two of these, *pbpB* (the gene for PBP3) and *pbpC* (a gene encoding a protein homologous to PBP3), actually encoded PBPs. Using these PCR fragments as probes, larger DNA fragments were obtained and sequenced, resulting in elucidation of

Table 1. Genetic characteristics of the genes investigated in this study

Gene*	Accession number		Identity of bacterial homologues (%)†	Hybridizing fragment	
	P. aeruginosa sequence	Bacterial homologue		*Spe*I	*Dpn*I
acoA^{H} (STS-163)	U15344	L35343 (*P. putida*)	71·1%	A	L
catR^{H} (STS-91)	U15402	M33817 (*P. putida*)	76·3%	P, Y	D
cypH^{H}	X84050	M28363 (*E. coli*)	63·4%	C	N
glpD	L06231	M55989 (*E. coli*)	72%	T	M
envA/ftsZ/ftsA	U19797	X55034 (*E. coli*)	65%/67%/45%	E	I
hemD (STS-50)	U15379	M74844 (*P. aeruginosa*)	99·6%	G	F_2
opdE	Z14064	–‡	–‡	L	C
oprD (STS-127)	U15326	X63152 (*P. aeruginosa*)	96·6%	F	A
oprF	M94078	–‡	–‡	L	C
oprH	M26954	–‡	–‡	M	A
oprO/oprP	M86648/X53313	–‡	–‡	C	N
pbpB/murE	X84053	K00137 (*E. coli*)	45·3%/44·6%	E	I
pbpC/soxR^{H}	–§	K00137 (*E. coli*)	40·7%/57·8%	Y	D
pheS^{H} (STS-74)	U15393	K02844 (*E. coli*)	74·5%	P	D
phoS^{H}	–‖	K01992 (*E. coli*)	–‖	G	F_2
proS^{H} (STS-87)	U15399	M97858 (*E. coli*)	72·5%	F	A
pyo (STS-103)	U15311	D12708 (*P. aeruginosa*)	98·2%	A	L
rpsB^{H} (STS-76)	U15394	X53651 (*S. platensis*)	74·8%	Q	B
sucC^{H}/*sucD*	X84052	J01619 (*E. coli*)	87·3%	Q	B
yhhF^{H}	X84051	U00039 (*E. coli*)	53·6%	H	E

* H as a superscript identifies a homologue of a gene from another species. Genes encode: *acoA*, acetoin dehydrogenase (Huang *et al.*, 1994); *catR*, homologue of the *catBC* operon activator (Rothmel *et al.*, 1990); *cypH*, homologue of cyclophilin (periplasmic peptidyl-prolyl *cis-trans*-isomerase) (Kawamukai *et al.*, 1989); *glpD*, membrane-associated glycerol-3-phosphate dehydrogenase (Schweizer & Po, 1994); *envA*, cell division gene (Beall *et al.*, 1988); *ftsZ*, cell division gene, putative involvement in septum initiation (Beall *et al.*, 1988); *ftsA*, cell division gene (Robinson *et al.*, 1984); *hemD*, homologue of uroporphyrinogen-III cosynthase (Mohr *et al.*, 1994); *opdE*, OprD expression regulatory locus (Huang *et al.*, 1992); *oprD*, outer-membrane imipenem and basic amino acid specific protein OprD (= D2) (Huang *et al.*, 1992); *oprF*, major outer-membrane protein OprF (= F) (Woodruff *et al.*, 1986); *oprH*, outer-membrane protein OprH (= H1) (Bell & Hancock, 1989); *oprO*, outer-membrane polyphosphate-specific porin OprO (Siehnel *et al.*, 1992); *oprP*, outer-membrane phosphate-specific porin OprP (= P) (Siehnel *et al.*, 1990); *pbpB*, PBP3 (Nakamura *et al.*, 1983); *murE*, UDP-*N*-acetylmuramyl tripeptide synthetase (Michaud *et al.*, 1990); *pbpC*, PBP3A (Nakamura *et al.*, 1983); *soxR*, homologue of the superoxide response regulator gene of *E. coli* (Amabile-Cuevas & Demple, 1991); *pheS*, phenylalanine-tRNA synthetase (Miller, 1984); *phoS*, periplasmic phosphate-binding protein (Burland *et al.*, 1993); *proS*, propyl-tRNA synthetase (Eriani *et al.*, 1990); *pyo*, homologue of structural gene for pyocin S2 (Sano *et al.*, 1993); *rpsB*, homologue of ribosomal protein small-subunit protein S2 of *S. platensis* (Sanangelantoni *et al.*, 1990); *sucC*, homologue of succinate dehydrogenase subunit A of *E. coli* (Buck *et al.*, 1985); *sucD*, homologue of succinate dehydrogenase subunit B of *E. coli* (Buck *et al.*, 1985); *yhhF*, homologue of *yhhF* gene of unknown function in *E. coli* (formerly called *ftsS*) (Sofia *et al.*, 1994).

† Identity refers to amino acid sequence identity for genes and nucleotide sequence identity for STS. Lengths of DNA sequences (in nucleotides) compared for the STS were: *acoA*^{H}, 274; *catR*^{H}, 276; *hemD*, 269; *oprD*, 294; *pheS*^{H}, 273; *proS*^{H}, 246; *pyo*, 279; *rpsB*, 375.

‡ No highly homologous genes; the C-terminus of *oprF* is 31% identical to that of *E. coli ompA*.

§ Submitted, but no accession number obtained yet.

‖ Not sequenced.

the entire sequences of both genes and most of the sequence of the *murE* gene downstream of *pbpB* and the *sox*R-homologue transcribed in the opposite direction to *pbpC*. Details of these genes will be presented elsewhere. Three other segments were found to be amplified by the same primers. All three were sequenced and found to contain nucleotide sequences homologous to the primers but often in different reading frames, such that the presumed product contained either one or none of the SXXK and KTG motifs that had been used to design the degenerate primers. Each of the sequences was used to perform a Geninfo Blast Network search using the National Center for Biotechnology Information service, which employs a basic alignment search tool to search available protein sequences. All six possible reading frames were searched for each sequence. One of the sequences cloned in plasmid pXL8 (plasmid pTZ8U containing a 510 bp insert) showed 87·3% identity to residues 239–388 from the *sucC* gene encoding the β-subunit of succinyl-CoA synthetase of *E. coli* and 63%, 53% and 50% identity with the equivalent subunit from *Trichomonas vaginalis*, pig and *Thermus aquaticus*, respectively. The third nucleotide of the TAA stop codon was the first nucleotide of the methionine codon of a stretch encoding 19 amino acids, 14 of which were identical to those encoded by the *E. coli sucD* gene which encodes subunit A of succinyl-CoA synthetase and is found in a similar location relative to the *sucC* gene of *E. coli*. The % G+C of the third position of codons was 84%, which was typical of a high G+C organism such as *P. aeruginosa* (West & Iglewski, 1988).

A second sequence cloned in plasmid pXL5 (plasmid pTZ18U containing a 380 bp insert) demonstrated 53·6% identity to amino acids 1–104 of a gene called ORF4, or *yhbF* from *E. coli*. A third sequence cloned into plasmid pXL12 (plasmid pTZ18U containing a 460 bp insert) demonstrated 63·4% identity to residues 24–176 of an *E. coli* gene encoding a periplasmic peptidyl-propyl *cis-trans*-isomerase ('rotamase') for which the names *rot* and *cypH* have been proposed. We prefer the latter due to the homology of this gene to cyclophilin from mammalian cells. These sequences also had a high G+C content (83% and 90% for *yhbF* and *cypH*, respectively) in the third codon position.

Part of the *ftsZ* gene was amplified using a set of primers described in Methods. The DNA sequence of the cloned 220 bp PCR product shared 60% identity to the *E. coli ftsZ* gene whereas the deduced amino acid sequence demonstrated a certain degree of identity with all known FtsZ proteins; the highest identity value was 65% with the *E. coli* homologue. A *P. aeruginosa* chromosomal DNA library constructed in λSE6 was screened by plaque hybridization using the 220 bp $ftsZ_{pao}$ PCR product, permitting cloning of the entire *ftsZ* gene on a 2100 bp sequence. Details of this cloning experiment will appear elsewhere. Sequence analysis revealed three potential ORFs of 235, 394 and a truncated protein of 42 amino acids. A computer search revealed 55% and 67% amino acid sequence identity of ORF394 with the *B. subtilis* and *E. coli* FtsZ and 45% identity between ORF235 and *E. coli* FtsA. We also noted 65% identity between the *E. coli* EnvA protein and the truncated ORF42 summarized in Table 1.

Mapping of other genetic markers

P. aeruginosa genomic DNA was digested with *Eco*RI and *Mbo*I and the digested fragments were separated by agarose gel electrophoresis. A directional cloning library was constructed in pTZ18R by electroelution of DNA fragments of 4–7 kb. We reasoned that this approach would decrease the bias of cloning small DNA fragments and would limit non-contiguous DNA sequences and other cloning artifacts. Analysis of plasmid DNA from 250 clones purified as white colonies on solid media containing IPTG and X-Gal revealed that 75% had inserts of 1·5–7 kb. Single-stranded DNA was prepared from 5 ml cultures, and sequencing reactions were performed on 115 clones with the universal and reverse primers. Only 16 of the more than 230 reactions resulted in no readable data. For positive autoradiograms, the sequence was read in one orientation. The data were entered manually in the University of Wisconsin Genetics Computer Group program SeqEd, and Dear and Staden (1992) and the BLAST (GCG version 8) programs were used to compare all sequences with one another. We noted only two clones that contained identical sequences, indicating that redundancy of sequence data was minimal and accuracy was assumed to be approximately 95%. The length of each sequence analysed varied from 172 bp to more than 450 bp. Sequences homologous to the pTZ18R vector and ambiguous data were not considered. Results for eight of these sequences (STS) are summarized in Table 1. The largest number of matches were found with a *Pseudomonas* sp. and with *E. coli*. The *P. aeruginosa* STS had identity values of between 45% and 99% with genes such as *acoA* (from *Pseudomonas putida*), *cat*R (from *P. putida*), *hemD*, *pyo*, *oprD* (from *P. aeruginosa*), *pheS* (from *E. coli*), *proS* (from *E. coli*), and *rpsB* (from *Spirulina platensis*).

Inspection of sequence databases and of the known mapped genes of *P. aeruginosa* indicated that additional genes could be mapped using specific intragenic oligonucleotide probes. A collection of 16 oligonucleotide probes was synthesized and used as *P. aeruginosa* gene probes as listed in Table 2.

In addition to the above genes, we selected six other clones previously obtained in our laboratories, namely, the structural genes for outer-membrane proteins OprF (Woodruff *et al.*, 1986), OprP (Siehnel *et al.*, 1988) and OprH (Bell & Hancock, 1989), the periplasmic phosphate-binding protein PhoS (Siehnel *et al.*, 1988) and *sn*-glycerol-3-phosphate dehydrogenase (GlpD), as well as the gene for a putative regulator of OprD expression, *opdE* (Huang *et al.*, 1992). This latter gene product, OpdE, was indicated by BLAST searching to be 40·9% identical to an *E. coli* ORF termed *yicM*, 30% identical to a chloramphenicol-resistance protein from *Streptomyces lividans* and 25% identical to the AraJ protein of *E. coli* and had a sequence that indicates it may be a membrane protein.

Table 2. Oligonucleotide probes used for mapping genes on the *P. aeruginosa* physical map

Gene*	Oligonucleotide sequence	Accession number	Hybridizing fragment	
			*Spe*I	*Dpn*I
ampC	5′-GTGACGCCGGAGACCCTGTTC-3′	X54719	A	L
ampR	5′-GCTCTCGCCCTCGTGGGTCAG-3′	X54719	A	L
aro-2	5′-TGATCCTCGTCGGCCCGATG-3′	L13865	C	N
dapB	5′-GGGCGAGGTGGTAGCCCAGGCG-3′	U04992	B	K†
envA	5′-ATGATCAAACAACGCACCTTGAAGAACATCATCCGGGCTAC-3′	U19797	E	I
groEL^{H}	5′-GTTAAGTTCGGCGATTCCGCT-3′	M63957	D	B
mexA	5′-AACGCCAGCCATGCGTGTACT-3′	L11616	H	E
oprH	5′-CATCGCCACCGCCCTGCTGGG-3′	M26954	M	A
oprI	5′-GAAGACGCAGCTGCTCGTGCT-3′	X58714	V	D
oprM	5′-AAGGGCAGGCCTACGGGCAGA-3′	L23839	H	E
oprP	5′-CGACATCGTGATCAAGACCAAG-3′	M86648	C	N
	5′-CCGGGACCGTGACCACCGACG-3′	Y00553	C	N
ponA	5′-GTTTCAGCGGCGCCTATCTCTAT-3′	L13867	B	K†
	5′-AGCTCTGCGTAACGTCCAGCT-3′	L13867		
qin	5′-CGCCACCCTGCTGGCCCTGGC-3′	L02105	F	A
rpoB	5′-GTCGCAGTTCATGGACCAGAA-3′	M99386	A	F_1
rpoH	5′-CTGACGATGACGCCGCAGCTG-3′	U09560	H	E
sodB	5′-CCGAGTTCGAAGGCAAGAGCCT-3′	L25675	E	I

* Genes encode: *ampC*, inducible type β-lactamase (previously *blaP*) (Lodge *et al.*, 1990); *ampR*, regulatory gene for *ampC* (may be identical to *blaJ*; Lodge *et al.*, 1990); *aro-2*, marker designed based on *aroK* of *E. coli* mapping close to the location of *aro-1* (Holloway *et al.*, 1994); *dapB*, dihydroxypicolinate reductase (Kwon *et al.*, 1994); *envA*, cell division gene; *groEL*, 60 kDa heat-shock-inducible protein, analogue of an *E. coli* chaperonin; *mexA*, inner-membrane antibiotic efflux protein, homologue of the *E. coli envC* gene (Poole *et al.*, 1993); *oprH*, outer-membrane protein OprH (Bell & Hancock, 1989); *oprI*, outer-membrane lipoprotein (Saint-Onge *et al.*, 1992); *oprM*, outer-membrane efflux protein OprM (formerly OprK) (Poole *et al.*, 1993); *oprP*, outer-membrane phosphate-specific porin OprP (Siehnel *et al.*, 1990); *ponA*, homologue of PBP1A (Martin *et al.*, 1993); *qin*, quinolone susceptibility; *rpoB*, β-subunit of RNA polymerase; *rpoH*, heat-shock response positive regulator (Benvenisti *et al.*, 1995); *sodB*, iron-cofactored superoxide dismutase (Hassett *et al.*, 1993). H as a superscript identifies a homologue of an *E. coli* gene.

† Although the *Dpn*I fragment is given as K, it is extremely difficult to differentiate this from the adjacent fragment, J.

All of the genes tested hybridized to unique and distinct *P. aeruginosa* chromosomal fragments obtained by *Spe*I and *Dpn*I digestion (Tables 1 and 2). The genes are shown on the physical/genetic map of *P. aeruginosa* in Fig. 1. An example of hybridization data of *Spe*I fragments of *P. aeruginosa* using *ampC*, *ampR* and *groEL* as probes is given in Fig. 2.

Correlates with known genes

Recently, a comprehensive review on genome mapping of *P. aeruginosa* PAO was published by Holloway *et al.* (1994). On this map, 60 genes were mapped with two enzymes (the details of the mapping for four of these appearing in this paper), and 95 were mapped with *Spe*I only. The data reported here increase the number of mapped genetic markers by 32, representing a 20% increase on those genes mapped previously. Eight genetic markers were mapped to known locations and thus served as positive controls for our genomic methods, namely *ampC* (previously *blaP*), *ampR* (previously *blaJ*), *oprF*, *oprI*, *ponA*, *qin*, *dapB* and *sodB* (*dapB* and *ponA* having only been mapped with *Spe*I previously). A further four genetic markers mapped at physical map locations near to related genes or the equivalent genetically mapped locations, namely *aro-2* (mapping near *aro-1*), *pheS* (mapping near a gene called *phe-2*), *hemD* [incorrectly placed in the Holloway *et al.* (1994) review but correctly placed in the original Mohr *et al.* (1994) manuscript] and *proS* (mapping near *proB*). Three genes were mapped using both large gene fragments and oligonucleotide probes, namely *envA*, *oprP* and *oprH*, and were found to map identically with both sets of probes. These findings corroborate the approach of physical mapping using oligonucleotide probes.

Two genetic markers mapped apart from their known mapping sites. A sequence, *pyo*, showing 98% identity to *pyoS2*, the structural gene for pyocin S2, mapped at a physical location of 26 min on the genetic map cf. 61 min for the *pyoS2* marker. The *aro-2* marker was identified using an *aroK*-specific probe but it mapped to the *Spe*I fragment C located more than 30 min from another gene with sequence homology to the *aroK* gene which mapped to *Spe*I fragment B. We conclude that these new genes probably represent homologues of the above-named

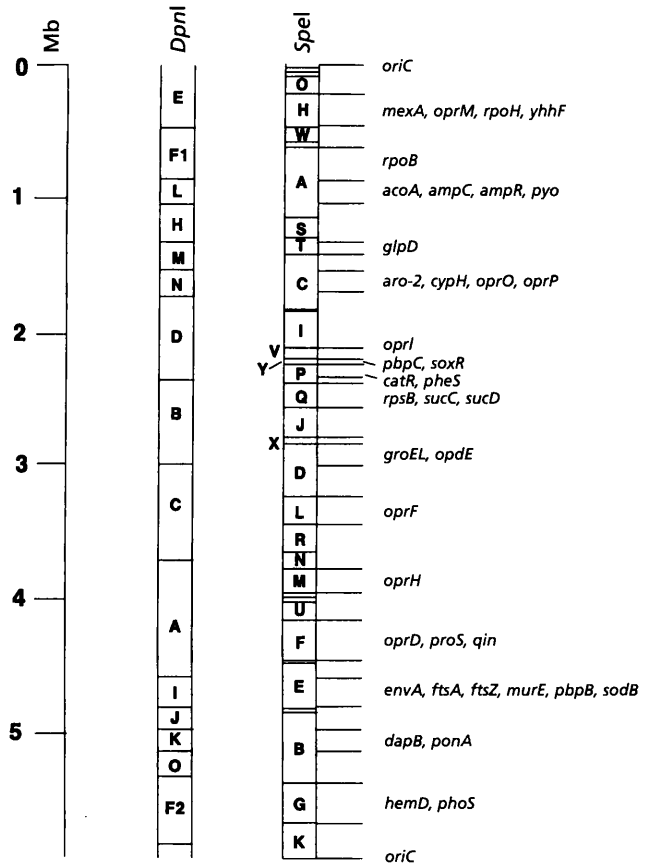

Fig. 1. Physical and genetic map of the 5·94 Mb genome of *P. aeruginosa* PAO1293. *Dpn*I and *Spe*I fragment sizes have been described previously and are identified in capital letters. *oriC* is the initial starting point on the map (Römling *et al.*, 1992; Holloway *et al.*, 1994). Details for genes mapped as cloned DNA fragments, PCR products, oligonucleotide or as STS are given in Tables 1 and 2. Smaller *Spe*I fragments are not shown.

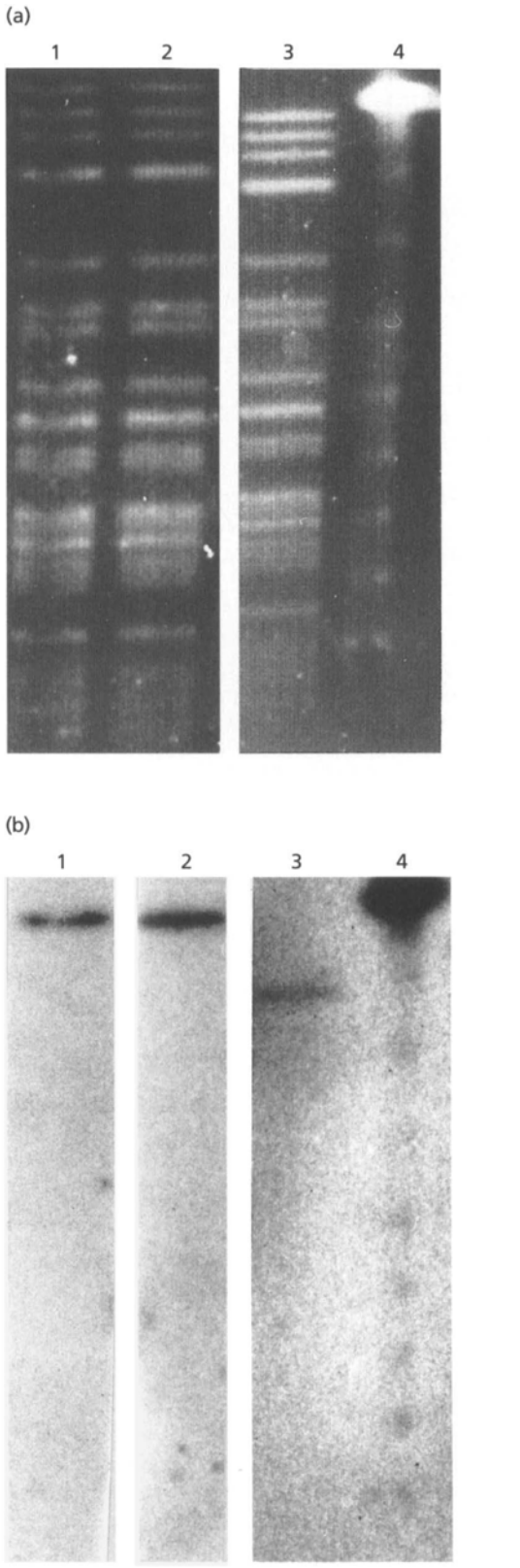

Fig. 2. (a) PFGE of *P. aeruginosa* PAO1293 DNA pre-digested with *Spe*I and (b) Southern-type gel hybridization using *ampC* (lane 1), *ampR* (lane 2) and *groEL* (lane 3) as ^{32}P-labelled oligonucleotide probes. Lane 4, phage λ DNA concatameric ladder.

genes, since several genes have been shown to be duplicated in *P. aeruginosa* (for example, of the genes mapped in the paper, *oprO/oprP* and *pbpB/pbpC* show strong homology). An alternative possibility is that the original genes were incorrectly mapped.

Another gene, *glpD*, was mapped based on hybridization with the cloned gene (Schweizer & Po, 1994). However it mapped more than 500 Mb from a marker called *glpD* (and assumed to represent the same gene) that was genetically located. The high homology of the cloned gene to the *E. coli glpD* gene (Schweizer & Po, 1994) and its location adjacent to other *glp* gene homologues (including *glpF*, *glpK* and *glpR*) (H. Schweizer, unpublished data) rather suggests that the cloned gene is the genuine *glpD* gene. In contrast, the previously described *glpD* gene (Cuskey & Phibbs, 1985) was only characterized based on the phenotype of a mutant, *glpD1*, which phenotype could not be complemented by our *glpD* clone (H. Schweizer, unpublished data). Thus we feel justified in redefining the location of the *glpD* gene.

Genes in *P. aeruginosa* that are functionally related are sometimes clustered. For example, we have separately cloned genes equivalent to ones found at the 5′- and 3′-ends of the major cell division operon of *E. coli*, namely *pbpB/murE* and *envA/ftsA/ftsZ*. These all mapped to the same fragments, indicating that they may also be genetically linked in *P. aeruginosa*. Similarly, the mapping of another gene, *phoS*, placed it in the same region of the chromosome as the *phoB* gene, the *E. coli* analogue of which can regulate expression of *phoS*, and the alkaline phosphatase (*phoA*) gene which is corregulated with *phoS*. In contrast, the corregulated *oprP* gene was more than 2 Mb distant.

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