Chromosome mapping in *Pseudomonas syringae* pv. *syringae* strain PS224

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A conjugation system for mapping the chromosome of *Pseudomonas syringae* pv. *syringae* PS224 has been developed using the IncP-10 plasmid R91-5; pMO22, a Tn501-loaded derivative of R91-5; and pMO75, R91-5 loaded with Tn5. Nine different donor origins were identified with R91-5 and pMO22. By insertion of Tn5 into various sites of the chromosome, an additional six donor origins were available using pMO75 as the donor plasmid. In all, 36 markers were located on three linkage groups. Many donor strains were unstable and the limited availability of stable donor strains has limited the extent to which markers have been located. This instability of donor strains is in marked contrast to the highly stable donor strains found in *P. putida* using the same plasmids. As in *P. aeruginosa* and *P. putida*, auxotrophic markers in *P. syringae* do not show the clustering of related markers found in enterobacteria.

Introduction

The genetic analysis of phytopathogenic bacteria provides an important approach to the understanding of virulence mechanisms and the most productive approach is the combination of chemical and transposon mutagenesis, the now classical techniques of conjugation and transduction mapping, and recombinant DNA analysis. This paper describes the chromosome mapping of *Pseudomonas syringae* pv. *syringae* PS224 using procedures which have been successfully developed for other species of *Pseudomonas*. The demonstrated need to adapt these existing procedures for *P. syringae* emphasizes the differences in response to plasmids and transposons which occur between species of *Pseudomonas*.

P. syringae pv. *syringae* PS224 was previously characterized with respect to a transduction system (Nordeen & Currier, 1983). In this paper we describe the use of the IncP-10 plasmid R91-5 and some of its derivatives for the development of a conjugation system in *P. syringae* pv. *syringae* PS224 and the construction of a chromosome map with 36 markers located.

Methods

Bacterial strains and plasmids. These are listed in Table 1.

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Abbreviation: Cma, chromosome-mobilizing ability.

Media and cultural conditions. Nutrient yeast broth (Y broth), nutrient agar, minimal medium and minimal agar have been previously described (Stanisich & Holloway, 1972). Stocks of amino acids, purines and pyrimidines (50 mM) were stored over chloroform and used at a final concentration of 100 µg ml⁻¹. When appropriate, media were supplemented with carbenicillin (500 µg ml⁻¹ for *P. aeruginosa*, 1000 µg ml⁻¹ for *P. syringae*; Pyopen, Beecham Australia), kanamycin (50 µg ml⁻¹ for *P. syringae*, 500 µg ml⁻¹ for *P. aeruginosa*; Sigma), streptomycin (100 µg ml⁻¹ for *P. syringae*, 500 µg ml⁻¹ for *P. aeruginosa*; Sigma) or rifampicin (200 µg ml⁻¹; Rimactane, Ciba-Geigy), nalidixic acid (100 or 500 µg ml⁻¹, Sigma). Nalidixic acid resistant mutants were first isolated using 100 µg ml⁻¹, but for some markers, particularly those less than 6 min from various donor origins, 100 µg ml⁻¹ was insufficient to stop the mating process. Other mutants were isolated which were resistant to more than 500 µg ml⁻¹ and were satisfactory as recipients for all donors tested. The higher-level mutants were referred to as NalA and the lower-level mutants as NalB (Hane & Wood, 1969). The location of the resistance loci was not determined. There was no indication that segregation of such resistance loci affected the mapping of any markers listed in Fig. 2.

Mutagenesis. Auxotrophs were isolated using either ethyl methane-sulphonate (EMS) (Watson & Holloway, 1976) or transposon mutagenesis with R91-5 loaded with Tn5 (Whitta *et al.*, 1985).

Interrupted mating. The method was modified from that of Dean & Morgan (1983). Overnight cultures of a nalidixic acid resistant recipient and a nalidixic acid sensitive donor in Y broth were centrifuged and resuspended in 0.9% (w/v) sodium chloride solution. Membrane filters 1 cm² in size (Gelman Metrical GA-6, 25 mm, Product no. 60172; or Hybond N nylon membrane filters, Amersham) were placed on absorbent pads (Gelman Absorbent Pad Kit, 47 mm, Product no. 66025) on a series of minimal agar plates. Prior to use, all filters were boiled in water for at least 5 min.

After the membranes and pads had been placed on the plates, the membranes were smoothed over with a glass spreader to ensure proper flow through to the absorbent pad. The donor and recipient

Table 1. *Bacterial strains and plasmids used in this study*

Strain	Genotype	Derivation or reference
<i>Pseudomonas syringae</i>		
PS224	Prototroph	Nordeen & Currier (1983)
PSA95	<i>pro-400</i>	EMS treatment of PS224
PSA502	<i>leu-403 pur-406 rif-200 trpE801::Tn5</i> (pMO75) (origin E)	Tn5 derivative of PSA1074
PSA503	<i>leu-403 pur-406 rif-200 cys-800::Tn5</i> (pMO75) (origin G)	Tn5 derivative of PSA1074
PSA507	<i>leu-403 pur-406 rif-200 his-801::Tn5</i>	Tn5 derivative of PSA1074
PSA508	<i>leu-403 pur-406 rif-200 argC800::Tn5</i> (pMO75) (origin O)	Tn5 derivative of PSA1074
PSA511	<i>leu-403 pur-406 rif-200 his-802::Tn5</i>	Tn5 derivative of PSA1074
PSA512	<i>leu-403 pur-406 rif-200 argH801::Tn5</i>	Tn5 derivative of PSA1074
PSA513	<i>leu-403 pur-406 rif-200 cys-801::Tn5</i> (pMO1896) (origin F)	Tn5 derivative of PSA1074
PSA602	<i>leu-403 pur-406 rif-200 trpE801::Tn5 nalB201</i>	Nal ^r derivative of PSA502
PSA605	<i>leu-403 pur-406 rif-200 cys-800::Tn5 nalB202 nalA402</i>	Nal ^r derivative of PSA503
PSA608	<i>leu-403 pur-406 rif-200 his-801::Tn5 nalB204 nalA403</i>	Nal ^r derivative of PSA507
PSA610	<i>leu-403 pur-406 rif-200 argC800::Tn5 nalB205 nalA404</i>	Nal ^r derivative of PSA508
PSA611	<i>leu-403 pur-406 rif-200 pro-800::Tn5 nalB206</i>	Nal ^r derivative of PSA509
PSA612	<i>leu-403 pur-406 rif-200 pro-800::Tn5 nalB206 nalA405</i>	Nal ^r derivative of PSA611
PSA614	<i>leu-403 pur-406 rif-200 cys-802::Tn5 nalB207 nalA406</i>	Nal ^r derivative of PSA510
PSA616	<i>leu-403 pur-406 rif-200 his-802::Tn5 nalB208 nalA407</i>	Nal ^r derivative of PSA511
PSA618	<i>leu-403 pur-406 rif-200 argH801::Tn5 nalB209 nalA408</i>	Nal ^r derivative of PSA512
PSA620	<i>leu-403 pur-406 rif-200 cys-801::Tn5 nalB210 nalA409</i>	Nal ^r derivative of PSA513
PSA780	<i>pur-409 trpC/D424 rif-214 nalB217 leu-803::Tn5</i>	Tn5 derivative of PSA1715
PSA786	<i>pur-409 trpC/D424 rif-214 nalB217 pyr-800::Tn5</i>	Tn5 derivative of PSA1715
PSA838	<i>pur-409 trpC/D424 rif-214 nalB217 argG819::Tn5</i>	Tn5 derivative of PSA1715
PSA846	<i>met-400 rif-215 pur-803::Tn5</i> (pMO75) (origin N)	Tn5 derivative of PSA205
PSA847	<i>met-400 rif-215 pob-800::Tn5</i>	Tn5 derivative of PSA205
PSA851	<i>hisD402 pyrB414 rif-216 pur-800::Tn5</i> (pMO75) (origin P)	Tn5 derivative of PSA1108
PSA1000	<i>pro-400</i> (pMO22) (origin A)	Plasmid-carrying derivatives of PSA95
PSA1001	<i>pro-400</i> (R91-5) (origin B)	
PSA1002	<i>pro-400</i> (pMO22) (origin C)	
PSA1003	<i>pro-400</i> (pMO22) (origin D)	
PSA1008	<i>hisD402 met-412 R91-5</i> (origin I)	Plasmid-carrying derivatives of PSA1101
PSA1009	<i>hisD402 met-412 R91-5</i> (origin H)	
PSA1011	<i>hisD402 met-412 R91-5</i> (origin J)	
PSA1012	<i>hisD402 met-412 R91-5</i> (origin K)	
PSA1013	<i>hisD402 met-412 R91-5</i> (origin L)	
PSA1074	<i>leu-403 pur-406 rif-200</i>	Rif ^r derivative of PSA1070
PSA1100	<i>hisD402 pyrB414</i>	EMS treatment of PSA29
PSA1101	<i>hisD402 met-412</i>	EMS treatment of PSA29
PSA1212	<i>hisD402 trpA419 leu-425 nalB219</i>	Nal ^r derivative of PSA1211
PSA1370	<i>trpF411 pur-407 leu-424</i>	EMS treatment of PSA1103
PSA1371	<i>trpF411 pur-407 leu-424 nalB218</i>	Nal ^r derivative of PSA1370
PSA1375	<i>trpF411 pur-408 met-411 leu-422 nalB211 nalA410</i>	Nal ^r derivative of PSA1373
PSA1535	<i>hisD402 pyrB414 met-419 nalB212</i>	Nal ^r derivative of PSA1203
PSA1540	<i>hisD402 pyrB414 argA413 nalB213</i>	Nal ^r derivative of PSA1205
PSA1550	<i>hisD402 pyrB414 ser-400 nalB214</i>	Nal ^r derivative of PSA1206
PSA1555	<i>hisD402 pyrB414 argF406 nalB215</i>	Nal ^r derivative of PSA1204
PSA1558	<i>hisD402 pyrB414 trpB420 nalB216</i>	Nal ^r derivative of PSA1557
PSA1595	<i>hisD402 met-412 ilvD404 pyrE424 nalB220</i>	Nal ^r derivative of PSA1307
PSA1700	<i>his-400 pur-409 trpC/D424</i>	EMS treatment of PSA1200
PSA1721	<i>his-400 pur-409 trpC/D424 nalB217 nalA411</i>	Nal ^r derivative of PSA1720
<i>P. aeruginosa</i>		
PAO5	<i>trp-54 rif-5</i>	Chandler & Krishnapillai (1977)
PAO11	<i>trp-54 nalA7</i>	Haas & Holloway (1976)
PAO764	<i>trp-54 argA49::Tn5 rif-5</i>	Tn5 insert of PAO5
<i>Escherichia coli</i>		
HB101	F ⁻ <i>hsdS20 (hsdR hsdM) recA13 arg-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1 supE44 λ⁻ thi-1 leuB6</i>	Maniatis <i>et al.</i> (1982)
<i>Plasmids</i>		
R68.45	Cma Cb Km/Nm Tc Tra	R68-ECM (<i>argB</i> ⁺ section)
R91-5	Cma Cb Dps (PRD1, PR3, PR4) Tra	Derepressed mutant of R91; Chandler & Krishnapillai (1977)
pMO22	Cma Cb Hg Tra Dps (PRD1, PR3, PR4) Tra	R91-5::Tn501 derivative; Carrigan <i>et al.</i> (1978)
pMO75	Cma Cb Km Tra Dps (PRD1, PR3, PR4) Tra	R91-5::Tn5 derivative; Whitta <i>et al.</i> (1985)

Table 2. Auxotrophic mutants of *P. syringae* isofunctional with characterized markers of *P. putida* PPN or *P. aeruginosa* PAO

Identification was carried out by R prime complementation using plasmid donors constructed as described by Holloway (1978) for PAO and Bray *et al.* (1987) for PPN. The transfer of the primes used varied between 7×10^{-2} and 2×10^{-5} per donor cell.

<i>P. syringae</i> marker	Prime plasmid	<i>P. aeruginosa</i> PAO or <i>P. putida</i> PPN isofunctional marker	Origin of R prime
<i>trp-424</i>	pMO788	<i>trpC</i> or <i>trpD</i>	Bray <i>et al.</i> (1987)
<i>arg-800</i>	pMO788	<i>argC</i>	
<i>trp-411</i>	pMO1610	<i>trpF</i>	
<i>pur-408</i>	pMO1556	<i>pur-70</i>	Monash collection
<i>met-411</i>	pMO1898	<i>met-9011</i>	
<i>pur-407</i>	pMO1551	<i>pur-66</i>	This study
<i>leu-424</i>	pMO1551	<i>leu-8</i>	
<i>pyr-424</i>	pMO1554	<i>pyrE</i>	Monash collection
<i>ilv-404</i>	pMO746	<i>ilvD</i>	
<i>met-412, met-419</i>	pMO746	<i>met-28</i>	Morgan & Dean (1985)
<i>pyr-414</i>	pMO746	<i>pyrB</i>	
<i>arg-801</i>	pMO1554	<i>argH</i>	Monash collection
<i>arg-413</i>	pMO1571	<i>argA</i>	
<i>arg-406</i>	pMO778	<i>argF</i>	Morgan (1982)

suspensions were placed on the membranes by using a Pasteur pipette. Two drops of the recipient (about 0.06 ml) were added to the membranes and allowed to absorb, after which two drops of the donor suspension were added to start the mating. Membranes were transferred at the appropriate time interval into sterile Wasserman tubes containing 0.5 ml of minimal medium containing the appropriate concentration of nalidixic acid for the marker being mapped. The filters were then vortexed immediately for 20 s. All procedures for the interrupted matings, including the plating of the samples, were carried out at 28 °C. Following vortexing, the entire contents of each Wasserman tube was spread to dryness on appropriate selective media. The plates were then incubated at 28 °C for 72–96 h and scored.

Results

Our approach to the mapping of the *P. syringae* pv. *syringae* chromosome was essentially the same as that used by Watson & Holloway (1978) for the mapping of *P. aeruginosa* PAT. A series of multiply marked strains were isolated, plasmids were identified which would act as donors capable of giving time-of-entry data, then a series of matings were carried out to determine the linkage relationships of the markers available.

Isolation of multiply marked strains

Using EMS and Tn5 mutagenesis, a series of multiply marked strains were derived from the wild-type prototrophic strain; those used for the subsequent linkage experiments are listed in Table 1. Most markers introduced into these strains were auxotrophic. Identification of particular biosynthetic lesions was made by growth responses to intermediates of biosynthetic path-

ways; for example, *hisD* mutants could be identified by growth on histidine but failure to grow on histidinol. In addition, complementation testing with prime plasmids of *P. aeruginosa* or *P. putida* to identify isofunctional mutants was used where growth on intermediates did not give useful information (such primes have been derived using R68.45: Holloway, 1978; Bray *et al.*, 1987) (Table 2). While interspecific complementation to prototrophy by this method does not always enable precise identification of the biochemical lesion of the auxotroph being tested, it is frequently possible to differentiate mutants having the same requirement, and by a process of elimination to identify the lesion with a high degree of likelihood. Such tests are, however, no substitute for the identification of enzyme deficiencies to identify mutants accurately.

Identification of plasmids displaying chromosome-mobilizing ability (Cma)

It had previously been shown that R68.45 (Haas & Holloway, 1976) had only very low Cma in *P. syringae* PS224 (Currier & Morgan, 1982). Hence, in view of the success of derivatives of the IncP-10 plasmid R91-5 in promoting chromosome mobilization in *P. putida* (Dean & Morgan, 1983), it was decided to determine if R91-5 and various derivatives possessed Cma in *P. syringae* PS224.

P. aeruginosa strains carrying R91-5 and various transposon-loaded derivatives were mated to various *P. syringae* PS224 auxotrophic derivatives and carbeni-

cillin-resistant derivatives were recovered at a frequency of about 10^{-4} per donor cell. Such transconjugants do not transfer R91-5 (or the other derivatives) when mated to other *P. syringae* strains. This is directly comparable to the situation in *P. putida* (Morgan & Dean, 1985) and other Gram-negative bacteria as reviewed by Krishnapillai (1988). For *P. putida* about 90% of carbenicillin-resistant transconjugants carrying pMO22 have stable Cma (Dean & Morgan, 1983). In *P. syringae*, of about 2000 transconjugants carrying R91-5, about 2% showed Cma. For about the same number of pMO22 transconjugants, 10% displayed Cma.

From these transconjugants a range of donors were selected and tested for their stable ability to show Cma with different auxotrophic markers. The stability of the donors carrying R91-5 or pMO22 was examined with respect to plasmid markers and the Cma phenotype. Tests of up to 1000 colonies for retention of Cma showed that this phenotype was frequently lost irrespective of whether selection was made for Cb^r or Cb^r and Hg^r in the case of pMO22. Different donors varied in the degree to which either or both Cb^r and Hg^r were lost in the absence of carbenicillin and/or mercury. Cma can be lost on storage without loss of Cb^r or Hg^r . Hence, the donors used in the crosses described below have been selected for stability of Cma phenotype. As is the practice with Hfr strains of *E. coli*, preliminary tests to ensure retention of Cma were done before use of a donor strain in any interrupted mating. Different donors showed various patterns of recombination frequency, suggesting that each donor had a specific origin of chromosome transfer, as is the case with pMO22 in *P. putida* (Dean & Morgan, 1983). As interrupted mating data are superior for the relative location of markers and the construction of a comprehensive map, a selection of donors were tested for their ability to provide data of this type.

As is shown below, donors isolated in this way were suitable for mapping markers in only some regions of the chromosome due to the pattern of origins, so it became necessary to isolate and identify other types of donors. Chumley *et al.* (1979) showed in *Salmonella typhimurium* that if Tn10 became integrated into the chromosome, a chromosome-mobilizing plasmid carrying Tn10 mobilizes the chromosome from the site of the Tn10 integration. Krishnapillai *et al.* (1981) have demonstrated the same phenomenon in *P. aeruginosa* with Tn1. A similar approach was used in *P. syringae* to identify other origin sites, so as to enable mapping access to as much of the chromosome as possible.

A variety of Tn5 mutants have been isolated in *P. syringae* using the suicide vector pMO75 (Whitta *et al.*, 1985). It has been shown in *P. putida* (D. Strom, R. Bray, J. Petering & A. Morgan, unpublished) that pMO75 can mobilize chromosomal markers at high frequency, the

origins being located at the site of chromosomal Tn5 insertions. By matings between *P. aeruginosa* PAO strains carrying pMO75 and *P. syringae* auxotrophs with Tn5 inserts, donors were obtained in which pMO75 had apparently integrated at or very close to the Tn5 insertions. This was verified by Southern hybridization analysis (data not shown). Recovery of proximal markers using these donors ranged up to 10^{-1} per donor cell and the time of entry curves obtained with them were comparable to those using R91-5 and pMO22 donors. The donors prepared by these various approaches are included in the data in Tables 3 and 4.

P. putida PPN pMO75 donors are known to mobilize chromosomal markers in either the clockwise or the anticlockwise direction (D. Strom, R. Bray, J. Petering & A. Morgan, unpublished). In order to extend mapping of the *P. syringae* chromosome, other Tn5-loaded derivatives of R91-5 were constructed with Tn5 in the opposite orientation to that of Tn5 on pMO75. R91-5 was transferred to *P. aeruginosa* PAO764, which has Tn5 inserted chromosomally. This derivative was then mated to *P. aeruginosa* PAO11, selecting for transfer of kanamycin resistance by Tn5 insertion into R91-5. To determine if Tn5 had inserted in R91-5 in the opposite orientation to that found in pMO75, matings were carried out in *P. putida*, where the location of markers has been accurately determined by time of entry using pMO75 donors (D. Strom & A. Morgan, unpublished). One R91-5::Tn5 construct, pMO1896, was shown to mobilize the chromosome in *P. putida* in the opposite direction to that found with pMO75 and this donor plasmid has been used to locate markers on the *P. syringae* chromosome.

In any time of entry determination it is implicit that the chromosome is transferred only unidirectionally or at best predominantly unidirectionally. While it has not been possible to determine whether transfer was unidirectional for all the origins used, data are available to suggest that if bidirectional transfer is occurring, it does not distort the linkage data obtained. The relative frequencies of marker recovery using various R91-5, pMO22 and pMO75 donors are listed in Table 3.

Let us take two origins, O (13 min, linkage group 2) and E (8 min, linkage group 2). With markers distal to origin O, recovery of markers is found at significant frequencies (*trpE801*, 8 min, 2×10^{-3} per donor cell; *his-802*, 5 min, 1×10^{-6} per donor cell). However, with *trpC/D424*, which is proximal to origin O, no recovery ($< 1 \times 10^{-7}$) is found. Thus origin O transfers at significant frequency in only one direction. Similar results are found with origin E, which transfers *argC800* and *trpC/D424* but not *his-802*. Similar data in Table 3, and data from other crosses not listed here, support the view that the donors used transfer the chromosome unidirectionally.

Table 3. *Relative recombination frequencies of markers using various R91-5 and pMO22 donor strains*

Donor strains were plate mated with a range of auxotrophic recipients to determine markers that were proximal and distal to the respective donor transfer origins. Selection was for the auxotrophic markers indicated.

Donor	Origin	Recipient	Selected marker	Recombination frequency (per donor cell)
PSA1000 (pMO22)	A	PSA1558	<i>pyrB414</i>	1×10^{-4}
		PSA502	<i>trpE801</i>	1×10^{-6}
		PSA1375	<i>leu-422</i>	$< 10^{-7}$
PSA1001 (R91-5)	B	PSA1558	<i>trpB420</i>	1×10^{-3}
		PSA1558	<i>pyrB414</i>	2×10^{-6}
		PSA502	<i>trpE801</i>	$< 10^{-7}$
PSA1002 (R91-5)	C	PSA1375	<i>trpF411</i>	5×10^{-2}
		PSA1375	<i>leu-422</i>	1×10^{-5}
		PSA780	<i>leu-803</i>	$< 10^{-7}$
PSA1003 (pMO22)	D	PSA507	<i>his-801</i>	1×10^{-2}
		PSA1721	<i>trpC/D424</i>	1×10^{-4}
		PSA838	<i>argG819</i>	$< 10^{-7}$
PSA502 (pMO75)	E	PSA508	<i>argC800</i>	1×10^{-2}
		PSA1721	<i>trpC/D424</i>	5×10^{-5}
		PSA511	<i>his-802</i>	$< 10^{-7}$
PSA513 (pMO1896)	F	PSA503	<i>cys-800</i>	1×10^{-4}
		PSA838	<i>argG819</i>	2×10^{-6}
		PSA780	<i>leu-803</i>	$< 10^{-7}$
PSA503 (pMO75)	G	PSA838	<i>argG819</i>	5×10^{-3}
		PSA507	<i>his-801</i>	1×10^{-6}
		PSA513	<i>cys-801</i>	$< 10^{-7}$
PSA1009 (R91-5)	H	PSA513	<i>cys-801</i>	8×10^{-3}
		PSA780	<i>leu-803</i>	2×10^{-4}
		PSA503	<i>cys-800</i>	$< 10^{-7}$
PSA1008 (R91-5)	I	PSA780	<i>leu-803</i>	7×10^{-3}
		PSA1375	<i>trpF411</i>	2×10^{-5}
		PSA513	<i>cys-801</i>	$< 10^{-7}$
PSA1011 (R91-5)	J	PSA503	<i>cys-800</i>	4×10^{-3}
		PSA838	<i>argG819</i>	1×10^{-5}
		PSA513	<i>cys-801</i>	$< 10^{-7}$
PSA1012 (R91-5)	K	PSA507	<i>his-801</i>	1×10^{-4}
		PSA838	<i>argG819</i>	5×10^{-5}
		PSA1721	<i>his-400</i>	$< 10^{-7}$
PSA1013 (R91-5)	L	PSA502	<i>trpE801</i>	9×10^{-4}
		PSA1721	<i>trpC/D424</i>	5×10^{-5}
		PSA1558	<i>trpB420</i>	$< 10^{-7}$

Strategy for obtaining time of entry data

In the first instance, donors of *P. syringae* carrying pMO22 or R91-5 were selected at random and, using plate matings with a variety of different auxotrophs, combinations of donors and auxotrophs were identified which gave high frequencies of recombinant prototrophs. Hence, it could be concluded that the origin sites of these donors were close to the markers involved. Then, using multiply marked strains containing these markers, plate matings were carried out to select recombinants whereby the coinheritance of the other markers could be measured. In the first series of such crosses, a process of trial and error was necessary, but as data accumulated, it was possible to become more selective in choosing combinations of donors and recipients with which time

of entry data enabled the relative positions of different markers to be measured. Typical time of entry data for pMO22 and R91-5 donors are shown in Fig. 1.

The origins of various donor strains have been denoted by letters. A series of crosses were set up and time of entry data obtained. The data showing the crosses used, the frequency of recombination of individual markers and the particular donor strains used to locate markers are summarized in Tables 3 and 4. From the times of entry obtained with these crosses it has been possible to construct a map in which the relative order of 36 markers has been determined. The data are insufficient to establish circularity of the map and three linkage groups have been established.

Taking the time of entry data for origins A and B, it is possible to order 11 markers on linkage group 1: *ser-400*,

Fig. 1. Time of entry curves. *P. syringae* PSA1000 (*pro-400*, pMO22) was mated with PSA1540 (*hisD402 pyrB414 argA413 nalB213*). The number of recombinants inheriting the wild-type allele of the donor is shown for each sampling time. ■, *pyrB414* (5 min); ▲, *hisD402* (7 min); □, *argA413* (9 min).

pyrB414, *met-419*, *hisD402*, *argF406*, *ilvD404*, *pyrE424*, *argA413*, *argH801*, *trpB420* and *trpA419*.

Three-factor crosses involving *pyrB414*, *met-419* and *hisD402* confirmed that *met-419* is the middle marker of these three (Table 5). By similar crosses, the order *pyrB414*–*hisD402*–*argA413* was established. Using the other donors, the relationships between additional markers and specific origins were established. The *leu-803* marker was located relative to origin sites I and H and *cys-801* found to be proximal to *leu-803* relative to origin H. The order of *his-801*, *cys-802*, *his-400*, *pur-409* and *trpC/D424* and their locations relative to origins D and H were established by time of entry. Linkage data (not shown) confirmed that *pur-409* was located close to *his-400* and the order given on the map reflects the time of entry data.

The range of origins provided by R91-5 and pMO22 donors was insufficient to allow the relationships of the above markers to be determined. However, donors

Fig. 2. Linkage map of *P. syringae* as obtained by time of entry data. Arrowheads show the origin site and direction of transfer for each of the donors used. ◁, pMO22 donor origins; ◀, R91-5 donor origins; ▽, ▿, pMO75 Tn5 Hfr origins (arrowheads indicate the direction of transfer). Origin sites A and B are located on linkage group 1, origin sites D, E, F, G, H, I, J, K, L and O on linkage group 2 and origin sites C, N and P on linkage group 3.

Table 4. Crosses used to obtain time of entry data by means of interrupted matings

Mating	Markers	Origin	Time of entry (min)	Linkage group
PSA1000 × PSA1540	<i>pyrB414</i>	A	5	1
	<i>hisD402</i>	A	7	1
	<i>argA413</i>	A	9	1
PSA1000 × PSA1550	<i>ser-400</i>	A	5	1
PSA1000 × PSA1595	<i>ilvD404</i>	A	8	1
PSA1000 × PSA1535	<i>met-419</i>	A	8	1
PSA1001 × PSA1540	<i>argA413</i>	B	5	1
	<i>hisD402</i>	B	8	1
	<i>pyrB414</i>	B	12	1
PSA1001 × PSA1212	<i>trpA419</i>	B	2	1
PSA1001 × PSA1558	<i>trpB420</i>	B	3	1
PSA1001 × PSA618	<i>argH801</i>	B	4	1
PSA1001 × PSA1595	<i>pyrE424</i>	B	5	1
PSA1001 × PSA1555	<i>argF406</i>	B	8	1
PSA1002 × PSA1375	<i>trpF411</i>	C	4	3
	<i>met-411</i>	C	4	3
	<i>pur-408</i>	C	6	3
	<i>leu-422</i>	C	11	3
	<i>pur-407</i>	C	11	3
PSA1002 × PSA1371	<i>leu-424</i>	C	11	3
	<i>his-801</i>	D	3	2
PSA1003 × PSA608	<i>cys-802</i>	D	4	2
PSA1003 × PSA614	<i>his-400</i>	D	9	2
	<i>pur-409</i>	D	10	2
	<i>trpC/D424</i>	D	14	2
PSA502 × PSA1721	<i>pur-409</i>	E	9	2
	<i>trpC/D424</i>	E	6	2
PSA513 × PSA605	<i>cys-800</i>	F	5	2
PSA503 × PSA838	<i>argG819</i>	G	10	2
PSA503 × PSA786	<i>pyr-800</i>	G	11	2
PSA1009 × PSA780	<i>leu-803</i>	H	14	2
PSA1009 × PSA620	<i>cys-801</i>	H	4	2
PSA1008 × PSA780	<i>leu-803</i>	I	5	2
PSA1011 × PSA605	<i>cys-800</i>	J	3	2
PSA1012 × PSA838	<i>argG819</i>	K	13	2
PSA1012 × PSA608	<i>his-801</i>	K	6	2
PSA1013 × PSA616	<i>his-802</i>	L	5	2
PSA1013 × PSA602	<i>trpE801</i>	L	8	2
PSA1013 × PSA610	<i>argC800</i>	L	13	2
PSA846 × PSA612	<i>leu-403</i>	N	1	3
PSA851 × PSA612	<i>leu-403</i>	P	4	3

formed by recombination between the Tn5 of pMO75 and a Tn5 insert into the chromosome of *P. syringae* were effective in establishing the linkage relationship of a variety of markers situated between *leu-803* and *his-802*. Fig. 2 shows the arrangement of 36 markers into three linkage groups, these data having been obtained by collectively using R91-5, pMO22 and pMO75/Tn5 insert donors. Attempts to find other donor sites to link these three linkage groups were not successful.

Discussion

The construction of a chromosome map of *P. syringae* using a variety of donors has demonstrated very clearly

the biological variability of plasmid response in the genus *Pseudomonas*. The IncP-10 plasmids pMO22 and pMO75 have been shown to form stable and highly effective donors in *P. putida* (Dean & Morgan, 1983; Morgan & Dean, 1985) and such donors have enabled a comprehensive and accurate chromosomal map of *P. putida* to be constructed. By contrast, in *P. syringae* the stability of IncP-10 donors was lower and the frequency of recombination less; consequently mapping by time of entry has been much more difficult. The use of Tn5 homology enabled a wider range of donors to be obtained and extension of this procedure may result in a more comprehensive map. The instability of the Cma phenotype of R91-5 derived donors in *P. syringae* PS224 was a problem not encountered in similar studies in *P. putida*.

Table 5. *Recombination frequency and linkage analysis with origin A (pMO22) and origin B (R91-5) donors*

PSA1000 (*pro-400*, pMO22) (origin A) is a transconjugant from the cross PAO1670 (pMO22) × PSA95 (*pro-400*). PSA1001 (*pro-400*, R91-5) (origin B) is a transconjugant from the cross PAO1670 (R91-5) × PSA95 (*pro-400*).

Selected marker(s)	Recombination frequency per donor	Coinheritance of unselected markers (%)*		
(a) <i>PSA1000 (pro-400, pMO22) (origin A) × PSA1535 (hisD402 pyrB414 met-419 nalB212)</i>				
		<i>pyrB414</i>	<i>met-419</i>	<i>hisD402</i>
<i>pyrB414</i> ⁺	1.6 × 10 ⁻⁵	—	89	16
<i>met-419</i> ⁺	2.8 × 10 ⁻⁶	86	—	26
<i>hisD402</i> ⁺	3.1 × 10 ⁻⁷	28	30	—
<i>met-419</i> ⁺ <i>pyrB414</i> ⁺	2.6 × 10 ⁻⁷	—	—	27
<i>pyrB414</i> ⁺ <i>hisD402</i> ⁺	1.7 × 10 ⁻⁷	—	96	—
<i>hisD402</i> ⁺ <i>met-419</i> ⁺	1.9 × 10 ⁻⁷	92	—	—
(b) <i>PSA1001 (pro-400, R91-5) (origin B) × PSA1540 (hisD402 pyrB414 argA413 nalB213)</i>				
		<i>pyrB414</i>	<i>hisD402</i>	<i>argA413</i>
<i>pyrB414</i> ⁺	4.5 × 10 ⁻⁶	—	42	39
<i>argA413</i> ⁺	3.9 × 10 ⁻⁴	0	20	—
<i>hisD402</i> ⁺	4.4 × 10 ⁻⁵	24	—	57
<i>argA413</i> ⁺ <i>pyrB414</i> ⁺	7.2 × 10 ⁻⁶	—	84	—
<i>pyrB414</i> ⁺ <i>hisD402</i> ⁺	4.3 × 10 ⁻⁷	—	—	76
<i>hisD402</i> ⁺ <i>argA413</i> ⁺	4.8 × 10 ⁻⁴	27	—	—

* One hundred recombinants for each selected marker were tested for coinheritance of unselected chromosomal markers by replica plating on appropriately supplemented media.

As pointed out by Holloway & Morgan (1986) for *P. aeruginosa* and *P. putida*, it is clear that certain groups of isofunctional markers show the same clustered arrangement in both species and it appears likely that a similar clustering occurs in *P. syringae*. Further mapping is needed to confirm this conservation of gene arrangement in *P. syringae*.

Given the recent success in using pulsed-field gel electrophoresis in establishing combined physical and genetic maps (Smith *et al.*, 1987, for *E. coli*; Holloway *et al.*, 1990, for *P. aeruginosa*) it seems that a similar approach for *P. syringae* based on the linkage data provided here would be most effective for constructing a more extensive chromosome map.

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