**REVIEW
ARTICLE****Genomic mapping of *Pseudomonas aeruginosa* PAO**B. W. Holloway,¹ U. Römling² and Burkhard Tümmler²

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¹Department of Genetics and Developmental Biology, Monash University, Clayton, Victoria 3168, Australia²Abteilung Biophysikalische Chemie, OE 4350 Medizinische Hochschule, Hannover D-30623, Hannover, Germany**Keywords:** *Pseudomonas aeruginosa*, genome mapping, genetic map, physical map**Background**

The extent of knowledge of genome arrangement in any bacterium is directly proportional to the degree of interest in the organism, its importance to medicine, agriculture, the environment and industry and the availability of means of genetic analysis. Until the introduction of recombinant DNA techniques, genome mapping was dependent on the availability and user-friendliness of systems of genetic exchange – conjugation, transduction and transformation being the most common. New DNA technologies have enabled the genetic mapping of those organisms for which *in vivo* systems of analysis are not available, and this topic has been recently reviewed (Smith & Condemine, 1990; Holloway, 1993). For *Pseudomonas aeruginosa*, a fortunate situation has been created by the availability of all three major systems of *in vivo* gene exchange, conjugation, transduction and transformation (Holloway & Morgan, 1986), as well as the newer technologies (Holloway *et al.*, 1992). Combined with an increasing research interest in this organism, mainly stimulated by its medical importance, the extent of genome mapping has increased markedly in the last few years.

The current situation is that there is a genetic map, constructed in the main from data obtained from conjugation and transduction (Holloway & Carey, 1993), and a physical map constructed using a combination of pulsed-field gel electrophoresis (PFGE), genome fragmentation by restriction enzymes that cut infrequently, and probing of the fragments so identified with cloned genes (Ratnaningsih *et al.*, 1990; Römling *et al.*, 1989, 1992; Römling & Tümmler, 1991).

In this paper, these separate maps have been updated and, as far as possible, correlations made between the data obtained by both mapping approaches. The distinction by which data are assigned to one or the other map is becoming more difficult. For example, new genes have

been identified in *P. aeruginosa* through sequence homology with known genes of other bacteria, and restriction maps have been constructed using frequently cutting enzymes, which has helped define the order of closely linked markers. Eventually, the two maps will become one, but at present, the data are best presented in the two formats.

The genetic map

Fig. 1 is a revision of the maps previously published in the sequence of volumes edited by O'Brien, the last published in 1993. Data have in the main been obtained by conjugation and transduction, and the conventions for indicating the location of markers is defined in the legend of Fig. 1. In Table 1 the data from both maps are assembled. The gene symbol and the phenotypic character, where known, are indicated as are the location on either or both maps, combined with selected references. For space considerations, the bibliography provided is focussed on mapping data, rather than other knowledge available about the marker, and references have been chosen where possible which will enable the reader to gain access to other information on the gene and its product.

The physical map

Construction of physical maps by PFGE enabled for the first time the complete and distinct representation of any bacterial genome in one experiment. A physical map consists of the pattern of rare-cutting restriction site landmarks dispersed on the chromosome. The construction of physical maps of now more than 50 bacteria has given enormous knowledge about the number and topology of replicons, location of genes on the chromosome and the genomic flexibility and structural conservation of a given species by comparative mapping

Fig. 1. Genetic map of *P. aeruginosa* PAO. The origin (0 min) of the genetic map has been arbitrarily designated at the *ilvB/C* locus. The following notation has been used. (i) Markers whose location is indicated by a bar joining the locus designation to the map have been located by interrupted matings using FP2, FP5, pMO514 or IncP-1 insert donors. (ii) A square bracket around a set of markers indicates that the gene order of the enclosed markers has been established. (iii) Curved lines indicate that the markers so joined are cotransducible using one or more of the bacteriophages F116, F116L, G101 and E79tv-2. (iv) The marker designation is underlined for those cases where there is evidence to locate the marker in the area in which the symbol is placed, but the relationships to flanking markers have not been determined.

Fig. 2. The physical map of *P. aeruginosa* PAO. The origin of the physical map has been designated at the *oriC* site. Colinearity of the physical and genetic maps has been achieved by locating the 14 min mark of the genetic map to one end of the *SpeI* AC fragment. Markers identified from sequence only are indicated by a dagger (†). An asterisk (*) indicates that the probe hybridizes with the adjacent fragments. While some attempt has been made to align markers mapped to individual restriction fragments, no conclusions can be drawn from the data in this figure with respect to the order of markers mapped physically, or their precise location on the genetic map (Fig. 1).

Table 1. Markers located on the genetic and physical maps of *P. aeruginosa* PAO (Figs 1 and 2)

References quoted mainly relate to the mapping of markers. For reasons of space, the enzyme fragments have been abbreviated in this Table to **S**, *SpeI* fragment, and **D**, *DpnI* fragment.

Gene symbol	Phenotypic trait affected	Genetic location (min)	Physical location (restriction fragment)	References
<i>absA</i>	Supersusceptibility to antibiotics	26		Angus <i>et al.</i> (1987)
<i>absB</i>	Supersusceptibility to antibiotics	19		Angus <i>et al.</i> (1987)
<i>aceA</i>	Pyruvate dehydrogenase (decarboxylase component)	6	S-B, D-J2	Jeyaseelan & Guest (1980); Jeyaseelan <i>et al.</i> (1980); this paper
<i>aceB</i>	Pyruvate dehydrogenase (dihydrolipoyl-transacetylase component)	6	S-B, D-J2	Jeyaseelan & Guest (1980); Jeyaseelan <i>et al.</i> (1980); this paper
<i>ada</i>	DNA repair gene for adaptive response to DNA methylating agents (using cloned <i>E. coli</i> gene)		S-H	Holloway <i>et al.</i> (1990); Tano <i>et al.</i> (1989)
<i>aglA</i>	General resistance to aminoglycosides	0		Mills & Holloway (1976)
<i>aglE</i>	High-level resistance to aminoglycosides	35		Holloway <i>et al.</i> (1979)
<i>agmR</i>	Activator for glycerol metabolism		S-D	Schweizer (1991); H. P. Schweizer (personal communication)
<i>aguA</i>	Agmatine deiminase	19		Haas <i>et al.</i> (1984)
<i>aguB</i>	N-Carbamylputrescine hydrolase	19		Haas <i>et al.</i> (1984)
<i>algA</i>	Phosphomannose isomerase-guanosine diphosphomannose pyrophosphorylase	34		Chitnis & Ohman (1993); May & Chakrabarty (1994)
<i>algB</i>	Regulation of alginate synthesis	13		Wozniak & Ohman (1991); Goldberg & Ohman (1987a)
<i>algC</i>	Phosphomannomutase	10	S-G	Zielinski <i>et al.</i> (1991); Shortridge <i>et al.</i> (1991)
<i>algD</i>	Guanosine diphosphomannose dehydrogenase	34	S-T, D-H	Deretic <i>et al.</i> (1987); Römling <i>et al.</i> (1989, 1992); Shortridge <i>et al.</i> (1991)
<i>algE</i>	Alginate transport function?	34		Chitnis & Ohman (1993)
<i>algF</i>	Alginate acetylase	34		Franklin & Ohman (1993)
<i>algG</i>	Alginate C5-mannuronan epimerase	34		Chitnis & Ohman (1990)
<i>algL</i>	Alginate lyase	34		Schiller <i>et al.</i> (1993)
<i>algP</i>	Regulation of alginate synthesis (also known as <i>algR3</i>)	10		Konyecsni & Deretic (1990)
<i>algQ</i>	Regulation of alginate synthesis (also known as <i>algR2</i>)	10		Konyecsni & Deretic (1990)
<i>algR</i>	Regulation of alginate synthesis (also known as <i>algR1</i>)	10	S-G, D-F2	Deretic <i>et al.</i> (1989); Römling <i>et al.</i> (1989); Shortridge <i>et al.</i> (1991)
<i>algU</i>	Genetic switch mechanism (also known as <i>algS</i> and <i>algT</i>)	68	S-F	Martin <i>et al.</i> (1993a); Shortridge <i>et al.</i> (1991)
<i>alg-8</i>	Polymerization function?	34		Darzins <i>et al.</i> (1985)
<i>alg-44</i>	Polymerization function?	34		Darzins <i>et al.</i> (1985)
<i>alg-60</i>	Not determined	34		Darzins <i>et al.</i> (1985)
<i>amiB</i>	Active transport system for amides	38		Wilson <i>et al.</i> (1993); Drew & Wilson (1992)
<i>amiC</i>	Regulatory gene for amidase	38		Wilson <i>et al.</i> (1993)
<i>amiE</i>	Structural gene for amidase	38		Day <i>et al.</i> (1975)
<i>amiR</i>	Regulatory gene for amidase	38		Day <i>et al.</i> (1975)
<i>amiS</i>	Active transport system for amides	38		Wilson <i>et al.</i> (1993); Drew & Wilson (1992)
<i>anr</i>	Anaerobic regulation of arginine deiminase and nitrate reduction	61	S-R	Galimand <i>et al.</i> (1991); Krishnapillai (1993)

Table 1 (cont.)

Gene symbol	Phenotypic trait affected	Genetic location (min)	Physical location (restriction fragment)	References
<i>ant-1</i>	Ability to use anthranilate as sole carbon source	46		Zhang <i>et al.</i> (1993a, b); Ratnaningsih <i>et al.</i> (1990)
<i>ant-2</i>	Ability to use anthranilate as sole carbon source	46		Zhang <i>et al.</i> (1993a, b); Ratnaningsih <i>et al.</i> (1990)
<i>ant-3</i>	Ability to use anthranilate as sole carbon source	46		Zhang <i>et al.</i> (1993a, b); Ratnaningsih <i>et al.</i> (1990)
<i>aphA</i>	Aminoglycoside 3'-phosphotransferase II	25		Okii <i>et al.</i> (1983)
<i>apr</i>	Alkaline protease		S-M	Shortridge <i>et al.</i> (1991)
<i>arcA</i>	Arginine deiminase	8		van der Wauven <i>et al.</i> (1984)
<i>arcB</i>	Catabolic ornithine carbamoyltransferase	8		van der Wauven <i>et al.</i> (1984)
<i>arcC</i>	Carbamate kinase	8		van der Wauven <i>et al.</i> (1984)
<i>arcD</i>	Arginine:ornithine exchange	8		Bourdineaud <i>et al.</i> (1993)
<i>argA</i>	N-Acetylglutamate synthase	8	S-G	Haas <i>et al.</i> (1977); O'Hoy & Krishnapillai (1987); Ratnaningsih <i>et al.</i> (1990)
<i>argB</i>	N-Acetylglutamate phosphotransferase	11	S-G	Haas <i>et al.</i> (1977); O'Hoy & Krishnapillai (1987); Ratnaningsih <i>et al.</i> (1990)
<i>argC</i>	N-Acetylglutamate semialdehyde dehydrogenase	23	S-AA	Haas <i>et al.</i> (1977); Ratnaningsih <i>et al.</i> (1990)
<i>argD</i> (= <i>aruC</i>)	N ² -Acetylornithine 5-aminotransferase	66		van der Wauven <i>et al.</i> (1988)
<i>argF</i>	Anabolic ornithine carbamoyltransferase	34	S-C	Haas <i>et al.</i> (1977); O'Hoy & Krishnapillai (1987); Ratnaningsih <i>et al.</i> (1990)
<i>argG</i>	Argininosuccinate synthase	34	S-C	Haas <i>et al.</i> (1977); Ratnaningsih <i>et al.</i> (1990)
<i>argH</i>	Argininosuccinate lyase	9	S-G	Haas <i>et al.</i> (1977); Ratnaningsih <i>et al.</i> (1990)
<i>argR9901</i>	Regulation of arginine utilization	1		Itoh & Matsumoto (1992)
<i>argR9902</i>	Regulation of arginine utilization	1		Itoh & Matsumoto (1992)
<i>argR9906</i>	Regulation of arginine utilization	66		Itoh & Matsumoto (1992)
<i>aroB</i> †	Aromatic amino acid biosynthesis		S-B	Martin <i>et al.</i> (1993c)
<i>aroK</i> †	Aromatic amino acid biosynthesis		S-B	Martin <i>et al.</i> (1993c)
<i>aro-1</i>	Aromatic amino acid requirement	39	S-C	Ratnaningsih <i>et al.</i> (1990)
<i>aruA</i>	Arginine succinyltransferase	66		Jann <i>et al.</i> (1986); van der Wauven <i>et al.</i> (1988); Y. Itoh (personal communication)
<i>aruB</i>	Succinylarginine dihydrolase	66		Jann <i>et al.</i> (1986); van der Wauven <i>et al.</i> (1988); Y. Itoh (personal communication)
<i>aruC</i> (= <i>argD</i>)	N ² -Succinylornithine 5-aminotransferase	66		van der Wauven <i>et al.</i> (1988); Y. Itoh (personal communication)
<i>aruD</i>	Succinylglutamate semialdehyde dehydrogenase	66		van der Wauven <i>et al.</i> (1988); Y. Itoh (personal communication)
<i>aruE</i>	N-Succinylglutamate desuccinylase	66		van der Wauven <i>et al.</i> (1988); Y. Itoh (personal communication)
<i>att</i> φCTX (= <i>attB</i>)	Attachment site for phage φCTX. This locus lies within a serine t-RNA gene		S-P	Hayashi <i>et al.</i> (1993)
<i>attH90</i>	Insertion site for prophage H90	2		Carey & Krishnapillai (1974)
<i>attJ51</i>	Insertion site for prophage J51	5		Carey & Krishnapillai (1975)

Table 1 (cont.)

Gene symbol	Phenotypic trait affected	Genetic location (min)	Physical location (restriction fragment)	References
<i>baw-9002</i>	Ability to utilize β -alanine as sole carbon and nitrogen source	6		Holloway & Matsumoto (1984)
<i>baw-9005</i>	Ability to utilize β -alanine as sole carbon and nitrogen source	45		Holloway & Matsumoto (1984)
<i>ben-1</i>	Ability to utilize benzoate as sole carbon source	47	S-Q	Zhang <i>et al.</i> (1993a, b); Ratnaningsih <i>et al.</i> (1990)
<i>ben-2</i>	Ability to utilize benzoate as sole carbon source	47	S-Q	Zhang <i>et al.</i> (1993a, b); Ratnaningsih <i>et al.</i> (1990)
<i>ben-3</i>	Ability to use benzoate as sole carbon source	47	S-Q	Zhang <i>et al.</i> (1993a, b); Ratnaningsih <i>et al.</i> (1990)
<i>ben-4</i>	Ability to utilize benzoate as sole carbon source	47	S-Q	Zhang <i>et al.</i> (1993a, b); Ratnaningsih <i>et al.</i> (1990)
<i>ben-1508</i>	Ability to use benzoate as a sole carbon source	46	S-Q	Zhang <i>et al.</i> (1993a, b); Ratnaningsih <i>et al.</i> (1990)
<i>blaI</i>	Regulatory gene for β -lactamase	41		Matsumoto & Terawaki (1982)
<i>blaJ</i>	Operator gene for <i>blaP</i>	25		Matsumoto & Terawaki (1982)
<i>blaK</i>	Regulatory gene for β -lactamase	26		Matsumoto & Terawaki (1982)
<i>blaL</i>	Regulatory gene for β -lactamase	41		H. Matsumoto (personal communication)
<i>blaP</i>	β -Lactamase	25	S-A, D-L	Matsumoto & Terawaki (1982); Holloway <i>et al.</i> (1993)
<i>blsA</i>	Sensitivity to β -lactam antibiotics	20		Fyfe & Govan (1984)
<i>braA</i>	Component of transport systems LIV-I and LIV-II	42		Hoshino <i>et al.</i> (1983)
<i>braB307</i>	LIV-II transport system	54		Hoshino <i>et al.</i> (1983)
<i>braC310</i>	Altered binding protein for leucine, isoleucine, valine, alanine and threonine (LIVAT-BP)	61	S-U	Hoshino <i>et al.</i> (1983); Krishnapillai (1993)
<i>bra-311</i>	Component of LIV-I system	61		Hoshino <i>et al.</i> (1983)
<i>bra-312</i>	Component of LIV-I system	61		Hoshino <i>et al.</i> (1983)
<i>bra-314</i>	Component of LIV-I system	59		Hoshino <i>et al.</i> (1983)
<i>braZ</i>	Branched chain amino acid transport		S-D	Krishnapillai (1993)
<i>car-9</i> (= <i>car-9003</i>)	Carbamoyl-phosphate synthetase (glutamine)	1	S-B	Haas & Holloway (1978); O'Hoy & Krishnapillai (1987); Ratnaningsih <i>et al.</i> (1990); Y. Itoh (personal communication); Kwon <i>et al.</i> (1994)
<i>carB</i> (= <i>car-9002</i>)	Carbamoyl-phosphate synthetase	1		Früh <i>et al.</i> (1985); Itoh & Matsumoto (1992); Y. Itoh (personal communication)
<i>carA</i> (= <i>car-9003</i>)	Carbamoyl-phosphate synthetase	1		Früh <i>et al.</i> (1985); Itoh & Matsumoto (1992); Y. Itoh (personal communication); Matsumoto <i>et al.</i> (1981)
<i>catA</i>	Catechol 1,2-oxygenase	46	S-Q	Kukor <i>et al.</i> (1988); O'Hoy & Krishnapillai (1987); Zhang <i>et al.</i> (1993a, b); Ratnaningsih <i>et al.</i> (1992)
<i>catB</i>	Muconate lactonizing enzyme	46	S-Q	Kukor <i>et al.</i> (1988); Ratnaningsih <i>et al.</i> (1992)
<i>catC</i>	Muconolactone isomerase	46	S-Q	Kukor <i>et al.</i> (1988); Ratnaningsih <i>et al.</i> (1992)
<i>ccf</i> (= <i>denB</i>)	Cytochrome c-551		S-H	Arai <i>et al.</i> (1991); Krishnapillai (1993); Zennaro <i>et al.</i> (1993)
<i>chuA</i>	Choline dehydrogenase	12		Matsumoto <i>et al.</i> (1981)

Table 1 (cont.)

Gene symbol	Phenotypic trait affected	Genetic location (min)	Physical location (restriction fragment)	References
<i>cbuC</i> (<i>cbu-9012</i>)	Betaine-homocysteine methyltransferase	12		Matsumoto <i>et al.</i> (1981)
<i>cbuD</i>	Dimethylglycine dehydrogenase	12		Matsumoto <i>et al.</i> (1981)
<i>cbuE</i>	Sarcosine dehydrogenase	12		Matsumoto <i>et al.</i> (1981)
<i>clbB</i>	Membrane protein		S-E	Whitchurch <i>et al.</i> (1991); Hobbs <i>et al.</i> (1993)
<i>cnu-9001</i>	Carnosinase	58		Holloway & Matsumoto (1984)
<i>crc-5</i>	Catabolite repression control	11		Wolff <i>et al.</i> (1991)
<i>cys-50</i>	Requirement for cysteine or methionine	45		O'Hoy & Krishnapillai (1987); Royle <i>et al.</i> (1981)
<i>cys-54</i>	Requirement for cysteine or methionine	56	S-L	Royle <i>et al.</i> (1981); O'Hoy & Krishnapillai (1987); Ratnaningsih <i>et al.</i> (1990)
<i>cys-59</i>	Requirement for cysteine or methionine	69		Soldati <i>et al.</i> (1984); O'Hoy & Krishnapillai (1987)
<i>cysG1</i>	Requirement for cysteine or methionine		S-L	Rodley <i>et al.</i> (1994)
<i>dapB</i> [†]	Dihydroxypicolinate reductase		S-B	Kwon <i>et al.</i> (1994)
<i>dcu-9013</i>	Ability to use dicarboxylic acids (adipate, pimelate, suberate and azelate) as sole carbon source	43		Matsumoto <i>et al.</i> (1981)
<i>dcu-9041</i>	Ability to use dicarboxylic acids (adipate, pimelate, suberate and azelate) as sole carbon source	43		Matsumoto <i>et al.</i> (1981)
<i>dcu-9008</i>	Ability to use dicarboxylic acids (adipate, pimelate, suberate and azelate) as sole carbon source	21		Matsumoto <i>et al.</i> (1981)
<i>dna-11</i>	Initiation of DNA synthesis	24		Herrmann <i>et al.</i> (1986)
<i>dnu</i>	D-Amino-acid oxidase (formerly <i>valD</i>)	7		Manoharan & Jayaraman (1978)
<i>eda-9001</i>	Entner-Doudoroff aldolase (2-keto-3-deoxy-6-phosphogluconate aldolase) (formerly designated as <i>hex</i>)	39	S-C	Roehl <i>et al.</i> (1983); Temple <i>et al.</i> (1990); Ratnaningsih <i>et al.</i> (1990)
<i>edd-1</i>	Entner-Doudoroff dehydratase (6-phosphogluconate dehydratase)	39	S-C	Cuskey & Phibbs (1985); Roehl <i>et al.</i> (1983); Temple <i>et al.</i> (1990); Ratnaningsih <i>et al.</i> (1990)
<i>elu-9001</i>	Ability to use ethanol	50		H. Matsumoto (personal communication)
<i>ent</i>	Outer-membrane enterobactin receptor		S-V, D-D	Farinha <i>et al.</i> (1993)
<i>eseA</i>	Phage E79 resistance	39		Holloway <i>et al.</i> (1979)
<i>eseB</i>	Phage E79 resistance	11		Holloway <i>et al.</i> (1979)
<i>exsCBAD</i> (= <i>exoR</i>)	Exoenzyme S production		S-L	Shortridge <i>et al.</i> (1991)
<i>exoS</i> (= <i>exoS</i>)	Exoenzyme S structural gene		S-A, D-H	D. Frank and others (personal communication)
<i>fbp</i>	Outer-membrane ferripyochelin binding protein		S-S, D-H	Farinha <i>et al.</i> (1993); Shortridge <i>et al.</i> (1991)
<i>fla</i> (1'ZW XY)	Flagella formation (<i>flaI</i>)	61		Tsuda & Iino (1983a, b); Tsuda <i>et al.</i> (1981)
<i>fla</i> (GCI HDBA FE)	Flagella formation (<i>flaII</i>)	59		Tsuda & Iino (1983a, b); Tsuda <i>et al.</i> (1981)
<i>fla</i>	Flagellin structural gene		S-U	Shortridge <i>et al.</i> (1991)
<i>fliA</i>	Minor sigma factor of RNA polymerase		S-N, D-C	Farinha <i>et al.</i> (1993)
<i>fliC</i>	Flagellin structural gene		S-U, D-A	Farinha <i>et al.</i> (1993)

Table 1 (cont.)

Gene symbol	Phenotypic trait affected	Genetic location (min)	Physical location (restriction fragment)	References
<i>fprA</i>	Ferripyoverdine uptake	47	S-J, D-B	Poole <i>et al.</i> (1993); I. Lamont (personal communication); this paper
<i>fsr</i>	4.5S RNA		S-R, D-C	Römling <i>et al.</i> (1992)
<i>ftpA</i>	Fe(III)-pyochelin regulator		S-A, D-F1	Ankenbauer & Quan (1994)
<i>fur</i>	Fe(III) uptake regulator		S-B, D-J/K	Prince <i>et al.</i> (1993); M. L. Vasil (personal communication)
<i>gap</i>	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase	39		Sage <i>et al.</i> (1993)
<i>gbu-9001</i>	Guanidinobutyrate amidinohydrolase	19		Holloway <i>et al.</i> (1979)
<i>gcu-1</i>	Ability to use glycine as sole carbon source	55	S-J	Zhang & Holloway (1992); Ratnaningsih <i>et al.</i> (1990)
<i>gcu-2</i>	Ability to use glycine as sole carbon source	45		Zhang & Holloway (1992)
<i>glk</i>	Glucokinase (formerly designated <i>glcK</i>)	39		Cuskey <i>et al.</i> (1985); Temple <i>et al.</i> (1990)
<i>glnA</i>	Glutamine synthetase	7		Janssen <i>et al.</i> (1982)
<i>gln-2020</i>	Glutamine synthetase	3		Janssen <i>et al.</i> (1982)
<i>gln-2022</i>	Regulation of nitrogen metabolism	7		Janssen <i>et al.</i> (1982)
<i>glpD</i>	Membrane-associated glycerol-3-phosphate dehydrogenase	38		Cuskey & Phibbs (1985)
<i>glt-1</i>	Glucose acting transport (formerly designated <i>glcT</i>)	39		Temple <i>et al.</i> (1990); P. V. Phibbs, Jr (personal communication); Cuskey <i>et al.</i> (1985)
<i>gnuK1</i>	Gluconokinase	48		W. Wallace & P. V. Phibbs, Jr (personal communication)
<i>gnuT1</i>	Gluconate active transport	48		W. Wallace & P. V. Phibbs, Jr (personal communication)
<i>gpn-9018</i>	Guanidinopropionate amidinohydrolase	18		Haas <i>et al.</i> (1984)
<i>gtu</i>	Ability to use glutarate as sole carbon source	10		Rahman & Clarke (1980)
<i>hemC</i> †	Porphobilinogen deaminase	9		Mohr <i>et al.</i> (1994)
<i>hemD</i> †	Uroporphyrinogen-III cosynthase	39		Mohr <i>et al.</i> (1994)
<i>hexC</i>	Regulation of hexose catabolic enzymes	39		Temple <i>et al.</i> (1990)
<i>hexR</i>	Regulation of hexose catabolic enzymes	39		Temple <i>et al.</i> (1990)
<i>hisI</i>	Requirement for histidine	69	S-E	O'Hoy & Krishnapillai (1987); Ratnaningsih <i>et al.</i> (1990); Mee & Lee (1967)
<i>hisIIA, B</i>	Requirement for histidine	7	S-G	O'Hoy & Krishnapillai (1987); Mee & Lee (1967); Ratnaningsih <i>et al.</i> (1990)
<i>hisIII</i>	Requirement for histidine	5		Holloway <i>et al.</i> (1979); Mee & Lee (1967)
<i>hisIV</i>	Requirement for histidine	4	S-B	O'Hoy & Krishnapillai (1987); Mee & Lee (1967); Ratnaningsih <i>et al.</i> (1990)
<i>hisV</i>	Requirement for histidine	19	S-H	Holloway <i>et al.</i> (1979); Mee & Lee (1967)
<i>hinH9051</i>	Histidase (formerly designated as <i>hut</i>)	7		Day <i>et al.</i> (1975); Holloway & Matsumoto (1984)
<i>hinU9070</i>	Urocanase	7		Day <i>et al.</i> (1975)

Table 1 (cont.)

Gene symbol	Phenotypic trait affected	Genetic location (min)	Physical location (restriction fragment)	References
<i>hii-109</i>	Ability to utilize histidine as sole carbon source	7		Day <i>et al.</i> (1975)
<i>hii-9089</i>	Ability to utilize histidine as sole carbon source	7		Holloway & Matsumoto (1984)
<i>hmuA9032</i>	Histaminase	17		Holloway & Matsumoto (1984)
<i>hmuC9027</i>	Imidazoleacetate hydroxylase	12		Holloway & Matsumoto (1984)
<i>hmu-9009</i>	Ability to utilize histamine as sole carbon source	17		Holloway & Matsumoto (1984)
<i>hmu-9001</i>	Ability to utilize histamine as sole carbon source	12		Holloway & Matsumoto (1984)
<i>hmu-9016</i>	Ability to utilize histamine as sole carbon source	12		Holloway & Matsumoto (1984)
<i>hmuF9025</i>	N-Formylglutamate deformylase	12		Holloway & Matsumoto (1984)
<i>hom</i>	Homoserine dehydrogenase	31		Sambanthamurthi <i>et al.</i> (1984); Clepet <i>et al.</i> (1992)
<i>hyu</i>	Utilization of L-hydroxyproline	7		Manoharan & Jayaraman (1979)
<i>ilvB</i>	Acetohydroxy acid synthase	0	S-B	O'Hoy & Krishnapillai (1987); Ratnaningsih <i>et al.</i> (1990)
<i>ilvC</i>	Acetohydroxy acid reductoisomerase	0	S-B	O'Hoy & Krishnapillai (1987); Ratnaningsih <i>et al.</i> (1990)
<i>ilvD</i>	Dihydroxy-acid dehydratase	20	S-H	O'Hoy & Krishnapillai (1987); Ratnaningsih <i>et al.</i> (1990)
<i>IS-P.A-1</i>	PAO insertion sequence – 2 copies		S-AH, S-A, D-H	Shortridge <i>et al.</i> (1991); Pritchard & Vasil (1990)
<i>kat</i>	Catalase production		S-E, D-J	D. Hassett & M. L. Vasil (personal communication)
<i>kauB</i>	4-Guanidinobutyraldehyde = 4-amino butyraldehyde	10		Jann <i>et al.</i> (1986)
<i>kgu-11</i>	Ability to use 2-ketogluconate as sole carbon source	49		W. Wallace & P. V. Phibbs, Jr (personal communication)
<i>lasA1</i>	Staphylolytic protease involved in elastase processing	51	S-L	Goldberg & Ohman (1987b); Kessler <i>et al.</i> (1993)
<i>lasB</i>	Structure gene for elastase		S-S	Shortridge <i>et al.</i> (1991)
<i>lasI</i>	Elastase regulation		S-K	Passador <i>et al.</i> (1993)
<i>lasR</i>	Elastase regulation		S-K	Shortridge <i>et al.</i> (1991); Passador <i>et al.</i> (1993)
<i>lesA</i>	Ability to be lysogenized	11		Miller & Ku (1978)
<i>lesB (= recA)</i>	Ability to be lysogenized	32		Miller & Ku (1978); Kokjohn & Miller (1988)
<i>leu-8</i>	Requirement for leucine	30	S-S	Royle <i>et al.</i> (1981); Ratnaningsih <i>et al.</i> (1990)
<i>leu-10</i>	Requirement for leucine	40	S-I	Royle <i>et al.</i> (1981); O'Hoy & Krishnapillai (1987); Filloux <i>et al.</i> (1989); Ratnaningsih <i>et al.</i> (1990); Römmling <i>et al.</i> (1992)
<i>lipA (= lip-1)</i>	Extracellular lipase structural gene	41	S-V, D-D	Holloway <i>et al.</i> (1993); Wohlfarth & Winkler (1988); Wohlfarth <i>et al.</i> (1992); this paper
<i>lipH (= lip-2)</i>	Extracellular lipase helper protein	41	S-V, D-D	Holloway <i>et al.</i> (1993); Wohlfarth & Winkler (1988); Wohlfarth <i>et al.</i> (1992); this paper
<i>lpp</i>	Outer-membrane protein		S-V	Krishnapillai (1993)
<i>lps</i>	Lipopolysaccharide biosynthesis	9		Goldberg <i>et al.</i> (1993)
<i>LPS A-band</i>	Lipopolysaccharide biosynthesis		S-K, D-F2	Lightfoot & Lam (1993)

Table 1 (cont.)

Gene symbol	Phenotypic trait affected	Genetic location (min)	Physical location (restriction fragment)	References
<i>LPSB-band</i>	Lipopolysaccharide biosynthesis		S-C, S-I, S-AI, D-D	Lightfoot & Lam (1993)
<i>lysA</i>	Diaminopimelate decarboxylase	10	S-G	O'Hoy & Krishnapillai (1987); Ratnaningsih <i>et al.</i> (1990)
<i>lys-9015</i>	Requirement for lysine	33		Holloway <i>et al.</i> (1979)
<i>mdlB</i>	Mandelate dehydrogenase	46		Rosenberg & Hegeman (1971)
<i>mdlC</i>	Benzylformate decarboxylase	46		Rosenberg & Hegeman (1971)
<i>met-26</i>	Requirement for methionine	19	S-H	Rodley <i>et al.</i> (1994)
<i>met-28</i>	Requirement for methionine	20	S-H	O'Hoy & Krishnapillai (1987); Ratnaningsih <i>et al.</i> (1990)
<i>met-29</i>	Requirement for methionine		S-L	Rodley <i>et al.</i> (1994)
<i>met-8003</i>	Requirement for methionine	20		O'Hoy & Krishnapillai (1987)
<i>met-9011</i>	Requirement for methionine	40	S-I	O'Hoy & Krishnapillai (1987); Filloux <i>et al.</i> (1989); Ratnaningsih <i>et al.</i> (1990); Römling <i>et al.</i> (1992)
<i>metC27</i>	Requirement for methionine		S-H	Rodley <i>et al.</i> (1994)
<i>min-9001</i>	Anucleated minicells	41		H. Matsumoto (personal communication)
<i>mms-13</i>	Sensitivity to methyl methane sulphonate, ATP-dependent DNase activity	11		Kung & Lee (1975); Lehrbach <i>et al.</i> (1980)
<i>mtu-9002</i>	Mannitol dehydrogenase and fructokinase	48		Royle <i>et al.</i> (1981); O'Hoy & Krishnapillai (1987)
<i>mtuD</i>	Mannitol dehydrogenase	48		Lessie & Phibbs (1984)
<i>mtuR</i>	Ability to utilize mannitol as sole carbon source	48		Lessie & Phibbs (1984)
<i>mucA</i> (= <i>muc-2</i>) (= <i>muc-22</i>)	Alginate genetic switch mechanism	68	S-F	Martin <i>et al.</i> (1993b); Fyfe & Govan (1983)
<i>mucB</i>	Alginate genetic switch mechanism	68	S-F	Martin <i>et al.</i> (1993b)
<i>muc-23</i>	Alginate genetic switch mechanism	69		Fyfe & Govan (1983)
<i>muc-25</i>	Alginate genetic switch mechanism	68		Fyfe & Govan (1983)
<i>muc-3739</i>	Alginate genetic switch mechanism	68		McGeorge <i>et al.</i> (1986)
<i>nadB</i>	Aspartate oxidase		S-F	Woodruff <i>et al.</i> (1992)
<i>nad-9003</i>	Requirement for nicotinamide	70		Holloway & Matsumoto (1984)
<i>nalA</i> (= <i>gyrA</i>)	Resistance to nalidixic acid and pipemidic acid	39		Rella & Haas (1982) Yoshida <i>et al.</i> (1990)
<i>nalB</i>	Low-level resistance to nalidixic acid, pipemidic acid, novobiocin and β -lactams	20		Rella & Haas (1982)
<i>narA</i>	Anaerobic growth with nitrate as electron acceptor	47		Jeter <i>et al.</i> (1984)
<i>narB</i>	Anaerobic growth with nitrate as electron acceptor	35		Jeter <i>et al.</i> (1984)
<i>narC</i>	Anaerobic growth with nitrate as electron acceptor	39		Jeter <i>et al.</i> (1984)
<i>narD</i>	Assimilatory nitrate reductase, dissimilatory nitrate reductase	47		Jeter <i>et al.</i> (1984)
<i>narE</i>	xanthine dehydrogenase Anaerobic growth with nitrate as electron acceptor	2		Jeter <i>et al.</i> (1984)
<i>nar-9001</i>	Reduction of nitrate to nitrite	30		Holloway & Matsumoto (1984)
<i>nar-9011</i>	Reduction of nitrate to nitrite	53		Holloway & Matsumoto (1984)
<i>nasA</i>	Assimilatory nitrate reductase and xanthine dehydrogenase	9		Jeter <i>et al.</i> (1984)

Table 1 (cont.)

Gene symbol	Phenotypic trait affected	Genetic location (min)	Physical location (restriction fragment)	References
<i>nasB</i>	Assimilatory nitrate reductase and xanthine dehydrogenase	15		Jeter <i>et al.</i> (1984)
<i>nasC</i>	Assimilatory nitrate reductase	9		Jeter <i>et al.</i> (1984)
<i>nfxB</i>	Norfloxacin resistance	0		Hirai <i>et al.</i> (1987)
<i>nfxC</i>	Norfloxacin resistance	47		Fukuda <i>et al.</i> (1990)
<i>nid</i> (= <i>denA</i>)	Nitrate reductase		S-H	Krishnapillai (1993); Arai <i>et al.</i> (1991); Zennaro <i>et al.</i> (1993)
<i>nir-9006</i>	Nitrite reduction	21		Matsumoto <i>et al.</i> (1981)
<i>ntmA</i>	Nitrogen metabolism	12		Jeter <i>et al.</i> (1984)
<i>ntmC</i>	Nitrogen metabolism	22		Jeter <i>et al.</i> (1984)
<i>oap-1</i>	Organic acid and alcohol permeability	45		Zhang & Holloway (1992)
<i>opdE</i>	Regulatory protein required for <i>oprD</i> expression		S-D, D-B	R. E. W. Hancock (personal communication)
<i>oprF</i>	Outer-membrane protein F		S-L, D-C	Römling <i>et al.</i> (1992)
<i>oprH</i>	Outer-membrane protein H		S-M	R. E. W. Hancock (personal communication)
<i>oprP</i>	Outer-membrane protein P		S-C, D-N	R. E. W. Hancock (personal communication)
<i>oprI</i>	Outer-membrane protein I		S-V	Römling <i>et al.</i> (1992)
<i>oriCI</i>	Chromosomal replication origin (<i>oriC</i> ₃₁₀)		S-K, D-E	Römling <i>et al.</i> (1992); Holloway <i>et al.</i> (1990)
<i>oriCII</i>	Chromosomal replication origin (<i>oriC</i> ₃₀₁)		S-K	Smith <i>et al.</i> (1991)
<i>oru-310</i> (= <i>oruII</i>)	Ornithine utilization	1		Y. Itoh (personal communication); Früh <i>et al.</i> (1985)
<i>oru-314</i> (= <i>oruIII</i>)	Ornithine utilization	40	S-I, D-D	Filloux <i>et al.</i> (1989); Früh <i>et al.</i> (1985); Römling <i>et al.</i> (1992); Y. Itoh (personal communication)
<i>pcaA</i>	Protocatechuate 3,4-oxygenase	16		Matsumoto <i>et al.</i> (1981)
<i>pcaB</i>	Carboxymuconate lactonizing enzyme	18		Kemp & Hegeman (1968)
<i>pcaD</i>	Enol-lactone hydrolase	18		Kemp & Hegeman (1968)
<i>pcaE</i>	β -Ketoadipate CoA-transferase	18		Kemp & Hegeman (1968)
<i>pft</i>	Pyocin F2 determinant genes	23		Shinomiya <i>et al.</i> (1983a, b)
<i>pgi-9001</i>	Phosphoglucosomerase	0		Lessie & Phibbs (1984); Holloway & Matsumoto (1984)
<i>phe-2</i>	Requirement for phenylalanine	38		Holloway & Matsumoto (1984)
<i>phe-3</i>	Requirement for phenylalanine	5	S-B	Holloway & Matsumoto (1984); Ratnaningsih <i>et al.</i> (1990)
<i>pbnAB</i>	Second anthranilate synthase involved in phenazine biosynthesis		S-U, D-A	Römling <i>et al.</i> (1989); Essar <i>et al.</i> (1990)
<i>pboA1</i>	Alkaline phosphatase		S-P, D-B	Holloway <i>et al.</i> (1990); Römling <i>et al.</i> (1992)
<i>pboA2</i>	Alkaline phosphatase		S-G, D-F2	Römling <i>et al.</i> (1992)
<i>pboB</i>	Positive regulatory element of <i>pbo</i> regulon (see <i>plcAB</i>)		S-G, D-F2	Shortridge <i>et al.</i> (1991); Römling <i>et al.</i> (1992); Holloway <i>et al.</i> (1990)
<i>pboS</i>	Periplasmic phosphate binding protein		S-G, D-F2	R. E. W. Hancock (personal communication)
<i>pilA</i> , B, C, D (<i>pilD</i> = <i>xcpA</i> , <i>pilA</i> = <i>fimA</i>)	Pilus biogenesis. <i>pilD</i> catalyses cleavage of propilin and N-methylation of N-terminal phenylalanine		S-E, D-I	Farinha <i>et al.</i> (1993); Whitchurch <i>et al.</i> (1991); Bally <i>et al.</i> (1992b); Strom <i>et al.</i> (1993)
<i>pilE</i>	Pilus biogenesis	71	S-E	Russell & Darzins (1994)
<i>pilG</i>	Pilus biogenesis	20	S-H, D-E	Darzins (1993, 1994)

Table 1 (cont.)

Gene symbol	Phenotypic trait affected	Genetic location (min)	Physical location (restriction fragment)	References
<i>pilH, I, J, K</i>	Pilus biogenesis	20	S-H	Darzens (1993, 1994); A. Darzens & P. L. Truax (personal communication)
<i>pilL</i>	Pilus biogenesis		S-E	P. R. Martin and others (personal communication)
<i>pilM, N, O, P</i>	Pilus biogenesis		S-B	Martin <i>et al.</i> (1993c)
<i>pilQ</i>	Pilus biogenesis		S-B	Martin <i>et al.</i> (1993c)
<i>pilR, S</i>	Pilus biogenesis		S-E, D-I	Shortridge <i>et al.</i> (1991); Farinha <i>et al.</i> (1993); Hobbs <i>et al.</i> (1993)
<i>pilT, U</i>	Pilus biogenesis	20	S-H, D-E	Whitchurch <i>et al.</i> (1991); P. R. Martin and others (personal communication)
<i>pilV</i>	Pilus biogenesis		S-E	P. R. Martin and others (personal communication)
<i>pip-6003</i>	Resistance to pipemidic acid	40		Rella & Haas (1982)
<i>plcA, B</i>	Regulation of phospholipase C (see <i>pboB</i>)	11		Gray & Vasil (1981a)
<i>plcN</i>	Non-haemolytic phospholipase C structural gene		S-C	Shortridge <i>et al.</i> (1991); Ostoff & Vasil (1987)
<i>plcSR1, R2</i>	Haemolytic phospholipase C structural gene and regulation (R1 and R2 are overlapping genes)	65	S-F	Pritchard & Vasil (1986); Shen <i>et al.</i> (1987); Shortridge <i>et al.</i> (1991)
<i>pobA</i>	<i>p</i> -Hydroxybenzoate hydroxylase	18		Matsumoto <i>et al.</i> (1981)
<i>pob-9063</i>	Ability to utilize <i>p</i> -hydroxybenzoate	54		Holloway & Matsumoto (1984)
<i>polA</i>	DNA polymerase	15		Lehrbach <i>et al.</i> (1976)
<i>ponA†</i>	Penicillin-binding protein		S-B	Martin <i>et al.</i> (1993c)
<i>proA</i>	Glutamate kinase	26	S-A	O'Hoy & Krishnapillai (1987); Ratnaningsih <i>et al.</i> (1990)
<i>proB</i>	Glutamylphosphate reductase	71		Krishna & Leisinger (1979); O'Hoy & Krishnapillai (1987)
<i>proC</i>	1-Pyrroline-5-carboxylate reductase	20	S-H	Krishna & Leisinger (1979); Holloway <i>et al.</i> (1979)
<i>priA-N</i>	Pyocin R2 determinant genes	22		Shinomiya <i>et al.</i> (1983a, b)
<i>priO</i>	Pyocin R2 determinant gene	23		Shinomiya <i>et al.</i> (1983a, b)
<i>priP</i>	Pyocin R2 determinant	24		Shinomiya <i>et al.</i> (1983a, b)
<i>priA, B</i>	Bifunctional proline dehydrogenase (PruA); 1-pyrroline-carboxylate dehydrogenase (PruB)	67	S-F	Soldati <i>et al.</i> (1984); Ratnaningsih <i>et al.</i> (1990)
<i>pts</i>	Phosphoenolpyruvate-fructose phosphotransferase	34		Roehl & Phibbs (1982)
<i>ptu-9001</i>	Ability to utilize putrescine as sole carbon source	11		Holloway & Matsumoto (1984)
<i>pur-66</i>	Requirement for purine	31	S-S	Holloway <i>et al.</i> (1979); Ratnaningsih <i>et al.</i> (1990)
<i>pur-70</i>	Requirement for purine	66	S-F	O'Hoy Krishnapillai (1987); Royle <i>et al.</i> (1981); Ratnaningsih <i>et al.</i> (1990)
<i>pur-136</i>	Requirement for purine	12	S-K	O'Hoy & Krishnapillai (1987); Ratnaningsih <i>et al.</i> (1990)
<i>pur-8001</i>	Requirement for purine	2	S-B	Tsuda <i>et al.</i> (1984); O'Hoy & Krishnapillai (1987); Ratnaningsih <i>et al.</i> (1990)
<i>pur-9013</i>	Requirement for purine	40	S-I	Matsumoto & Tazaki (1975); Ratnaningsih <i>et al.</i> (1990)
<i>puuA</i>	Adenine deaminase	16		Matsumoto <i>et al.</i> (1978)
<i>puuB</i>	Guanine deaminase	4		Matsumoto <i>et al.</i> (1978)

Table 1 (cont.)

Gene symbol	Phenotypic trait affected	Genetic location (min)	Physical location (restriction fragment)	References
<i>pnuC</i>	Xanthine dehydrogenase	57		Matsumoto <i>et al.</i> (1978)
<i>pnuD</i>	Uricase	57		Matsumoto <i>et al.</i> (1978)
<i>pnuE</i>	Allantoinase	57		Matsumoto <i>et al.</i> (1978)
<i>pnuF</i>	Allantoicase	58		Matsumoto <i>et al.</i> (1978)
<i>pvd</i>	Pyoverdine synthesis		S-F	Krishnapillai (1993)
<i>pvdA</i>	L-Ornithine <i>N</i> ⁵ -oxygenase	47		Ankenbauer <i>et al.</i> (1986); Visca <i>et al.</i> (1992, 1994); Rombel & Lamont (1992)
<i>pvdB</i>	Pyoverdine synthesis	47	S-J, D-B	Ankenbauer <i>et al.</i> (1986); Visca <i>et al.</i> (1992); this paper
<i>pvd-22</i>	Pyoverdine production	23		Hohnadel <i>et al.</i> (1986)
<i>pyc-2</i>	Pyruvate carboxylase	35		Roehl <i>et al.</i> (1983)
<i>pyoAP41</i>	Pyocin AP41 determinant	34		Sano & Kageyama (1984)
<i>pyoS1</i>	Pyocin S1 determinant	62		Sano <i>et al.</i> (1990)
<i>pyoS2</i>	Pyocin S2 determinant	62		Sano <i>et al.</i> (1990)
<i>pyrB</i>	Aspartate carbamoyltransferase	20	S-H	Holloway <i>et al.</i> (1979)
<i>pyrD</i>	Dihydroorotate dehydrogenase	41		Holloway <i>et al.</i> (1979)
<i>pyrE</i>	Orotidylic acid pyrophosphorylase	11	S-G	Holloway <i>et al.</i> (1993); Ratnaningsih <i>et al.</i> (1990)
<i>pyrF</i>	Orotidine-5'-monophosphate decarboxylase	41	S-V, D-D	Holloway <i>et al.</i> (1979); this paper
<i>pyr-81</i>	Requirement for pyrimidine	20	S-H	Ratnaningsih <i>et al.</i> (1990)
<i>pyr-85</i>	Requirement for pyrimidine	34		O'Hoy & Krishnapillai (1987)
<i>pyrX†</i>	Dihydroorotase		S-H	A. Darzins & P. L. Truax (personal communication)
<i>pyu-9010</i>	Dihydropyrimidinase	20		Matsumoto <i>et al.</i> (1981)
<i>qin</i>	Quinoline sensitivity		S-F, D-A	Römling <i>et al.</i> (1992)
<i>quu-9002</i>	Ability to utilize quinate or shikimate as sole carbon source	18		Holloway & Matsumoto (1984)
<i>recA</i>	Recombination function	32	S-T, D-H	Früh <i>et al.</i> (1983); Kokjohn & Miller (1988); Shortridge <i>et al.</i> (1991); Römling <i>et al.</i> (1989)
<i>recX</i>	Recombination function	32		Sano (1993)
<i>regA</i>	Regulation of <i>toxA</i> (see <i>toxR</i>)			
<i>regB</i> (= <i>rpoB</i>)	Regulation of <i>toxA</i>		S-F, D-A	Römling <i>et al.</i> (1992); Shortridge <i>et al.</i> (1991)
<i>rfb</i>	O-antigen production	39		J. B. Goldberg (personal communication)
<i>rifA</i>	Resistance to rifampicin	23		Holloway <i>et al.</i> (1979)
<i>rpoC</i>	β -Subunit of RNA polymerase		S-L	Holloway <i>et al.</i> (1990)
<i>rpoD</i>	Sigma 70 cofactor of RNA polymerase		S-W	Ronald <i>et al.</i> (1992)
<i>rpoN</i>	Sigma 54 cofactor of RNA polymerase		S-E, D-I	Farinha <i>et al.</i> (1993)
<i>rpoS</i>	Sigma 70 cofactor of RNA polymerase		S-T	Ronald <i>et al.</i> (1992)
<i>rrnA</i>	Ribosomal RNA		S-AA, D-F1	Römling <i>et al.</i> (1992)
<i>rrnB</i>	Ribosomal RNA		S-F, D-A	Römling <i>et al.</i> (1992)
<i>rrnC</i>	Ribosomal RNA		S-AF	Römling <i>et al.</i> (1992)
<i>rrnD</i>	Ribosomal DNA		S-G, D-F2	Römling <i>et al.</i> (1992)
<i>ser-3</i>	Requirement for serine	19	S-H	O'Hoy & Krishnapillai (1987); Ratnaningsih <i>et al.</i> (1990)
<i>ser-33</i>	Requirement for glycine or serine (= <i>gly-1</i>)	4	S-B	O'Hoy & Krishnapillai (1987); Ratnaningsih <i>et al.</i> (1990)
<i>sodA</i>	Manganese cofactored superoxide dismutase		S-E, D-I	Hassett <i>et al.</i> (1993)

Table 1 (cont.)

Gene symbol	Phenotypic trait affected	Genetic location (min)	Physical location (restriction fragment)	References
<i>sodB</i>	Iron cofactored superoxide dismutase		S-E, D-I	Hassett <i>et al.</i> (1993)
<i>somA</i>	O-serotypic specificity determinant	40		Matsumoto & Tazaki (1975); Holloway <i>et al.</i> (1979)
<i>somB</i>	Smooth to rough mutation	40		Matsumoto & Tazaki (1975); Holloway <i>et al.</i> (1979)
<i>srs</i>	Short rod-shaped organisms	57		Gotoh <i>et al.</i> (1984)
<i>ssr</i>	6S RNA		S-G, D-F2	Römling <i>et al.</i> (1992)
<i>sss</i>	Pyoverdinin regulatory gene		S-H	Krishnapillai (1993)
<i>strA</i>	Resistance to streptomycin	24		Holloway <i>et al.</i> (1979)
<i>thi-8001</i>	Requirement for thiamin	72		Tsuda <i>et al.</i> (1984)
<i>thr-48</i> (= <i>thrC</i>)	Requirement for threonine	31	S-S	O'Hoy & Krishnapillai (1987); Ratnaningsih <i>et al.</i> (1990); Clepet <i>et al.</i> (1992)
<i>thr-59</i> (= <i>hom</i>)	Requirement for homoserine	31	S-S	O'Hoy & Krishnapillai (1987); Ratnaningsih <i>et al.</i> (1990); Clepet <i>et al.</i> (1992)
<i>thr-60</i>	Requirement for homoserine and threonine or threonine and methionine	31	S-S	O'Hoy & Krishnapillai (1987); Ratnaningsih <i>et al.</i> (1990)
<i>thrB</i>	Complementation of <i>thrB</i> mutant of <i>E. coli</i>		S-K	Krishnapillai (1993); Clepet <i>et al.</i> (1992)
<i>tolA</i>	Tolerance to pyocin AR41	1		Mills & Holloway (1976)
<i>tolB</i>	Tolerance to pyocin AR41	66		Mills & Holloway (1976); Holloway <i>et al.</i> (1979)
<i>tolC</i>	Tolerance to pyocin AR41	3		Mills & Holloway (1976); Holloway <i>et al.</i> (1979)
<i>tolD</i>	Tolerance to pyocin AR41	14		Mills & Holloway (1976); Holloway <i>et al.</i> (1979)
<i>tolE</i>	Tolerance to pyocin AR41	30		Mills & Holloway (1976); Holloway <i>et al.</i> (1979)
<i>toxA</i> (= <i>toxA1</i>)	Exotoxin A structural gene	62	S-AH, D-A	Hanne <i>et al.</i> (1983); Römling <i>et al.</i> (1992); Shortridge <i>et al.</i> (1991)
<i>tox-1</i>	Exotoxin production	26		Gray & Vasil (1981b)
<i>tox-2</i>	Exotoxin production	23		Gray <i>et al.</i> (1982)
<i>tox-3</i>	Exotoxin production	62		Holloway & Matsumoto (1984)
<i>toxR</i> (= <i>regA</i>)	Regulatory element of <i>toxA</i> expression		S-F, D-A	Shortridge <i>et al.</i> (1991); Römling <i>et al.</i> (1992)
<i>tpsA</i>	Sensitivity to trimethoprim	19		Fyfe & Govan (1984)
<i>tRNA</i>	Cluster of tRNA genes: <i>tRNA^{Trp}</i> , <i>tRNA^{Trp}</i> (CCA); <i>tRNA^{Thr}</i> , <i>tRNA^{Thr}</i> (CCA); <i>tRNA^{Gly}</i> , <i>tRNA^{Gly}</i> (GGT); <i>tRNA^{Tyr}</i> , <i>tRNA^{Tyr}</i> (TCC); <i>tRNA^{Thr}</i> , <i>tRNA^{Thr}</i> (GTA)		S-A	Krishnapillai (1993); Hughes & Jones (1988)
<i>tRNA^{Val}</i>	<i>tRNA^{Val}</i> (TAC)	41		Bally <i>et al.</i> (1992a)
<i>tRNA^{Asp}</i>	<i>tRNA^{Asp}</i> (GTC)	41		Bally <i>et al.</i> (1992a)
<i>tRNA^{ser}</i>	See <i>attφCTX</i>			
<i>trpA</i>	Tryptophan synthase (subunit A)	14	S-AC, D-E	O'Hoy & Krishnapillai (1987); Römling <i>et al.</i> (1992)
<i>trpB</i>	Tryptophan synthase (subunit B)	14	S-AC, D-E	O'Hoy & Krishnapillai (1987); Römling <i>et al.</i> (1992)
<i>trpC</i>	Indole-3-glycerol-phosphate synthase	23	S-AA, D-F1	O'Hoy & Krishnapillai (1987); Ratnaningsih <i>et al.</i> (1990)
<i>trpD</i>	Anthranilate phosphoribosyltransferase	23	S-AA, D-F1	O'Hoy & Krishnapillai (1987); Ratnaningsih <i>et al.</i> (1990)

Table 1 (cont.)

Gene symbol	Phenotypic trait affected	Genetic location (min)	Physical location (restriction fragment)	References
<i>trpE</i>	Anthranilate synthase α -subunit	23	S-W, D-F1	Calhoun <i>et al.</i> (1973); Royle <i>et al.</i> (1981); Ratnaningsih <i>et al.</i> (1990); Römmling <i>et al.</i> (1992)
<i>trpF</i>	Phosphoribosylanthranilate isomerase	40	S-I, D-D	Calhoun <i>et al.</i> (1973); Royle <i>et al.</i> (1981); Filloux <i>et al.</i> (1989); Ratnaningsih <i>et al.</i> (1990); Römmling <i>et al.</i> (1992)
<i>trpG</i>	Anthranilate synthetase, β -subunit	23	S-AA, D-F1	Römmling <i>et al.</i> (1989); Essar <i>et al.</i> (1990)
<i>trpI</i>	Tryptophan synthase transcription activator	14	S-AC, D-E	Römmling <i>et al.</i> (1992); Chang & Crawford (1990)
<i>tyrA, B</i>	Ability to use tyramine as sole carbon or nitrogen source	48		Cuskey <i>et al.</i> (1987)
<i>tyu-9009</i>	Homogentisicase	50		Matsumoto <i>et al.</i> (1978)
<i>tyu-9030</i>	Ability to use tyrosine as sole carbon source	50		W. Wallace & P. V. Phibbs, Jr (personal communication)
<i>uvr-2003</i>	UV sensitivity	19		Früh (1984)
<i>val-2</i>	Requirement for valine	73		O'Hoy & Krishnapillai (1987)
<i>vtu-9001</i>	Vanillate demethylase	3		Holloway & Matsumoto (1984)
<i>xcb-1</i>	Exoproteins hyperproduction	41		Björklind <i>et al.</i> (1985)
<i>xcb-1</i>	Extracellular protein hyperproduction	41		Björklind <i>et al.</i> (1985)
<i>xcpA</i> (= <i>xcp-1</i> , = <i>pilD</i>)	Production or release of extracellular protein	70		Wretling & Pavlovskis (1984); Bally <i>et al.</i> (1989, 1992b)
<i>xcp-2</i>	Production of exoenzymes	23		Wretling & Pavlovskis (1984)
<i>xcp-3</i>	Production of exoenzymes	23		Wretling & Pavlovskis (1984)
<i>xcp-4</i>	Production of exoenzymes	26		Wretling & Pavlovskis (1984)
<i>xcp-6</i>	Release of exoproteins	47		Wretling & Pavlovskis (1984); Filloux <i>et al.</i> (1989)
<i>xcpPQRST</i> <i>UVWXYZ</i>	Release of exoproteins	40		Akrim <i>et al.</i> (1993); Filloux <i>et al.</i> (1989); Wretling & Pavlovskis (1984); Bally <i>et al.</i> (1992b)
<i>zwf-1</i>	Glucose-6-phosphate dehydrogenase	39		Roehl <i>et al.</i> (1983); Temple <i>et al.</i> (1990)

† Markers identified from sequence only.

of strains. In infection epidemiology genomic fingerprints have been shown to be universally applicable with a significant improvement of discrimination between strains when compared to other typing methods.

Two research groups independently created a physical map of the 5.9 Mbp large circular chromosome of *P. aeruginosa* PAO. Both groups used the restriction enzyme *SpeI*. Römmling and coworkers used *DpnI* (Römmling *et al.*, 1989) and later on *SmaI* and *PaeI* as additional restriction enzymes (Römmling & Tümmeler, 1992). One map was mainly based on hybridization data (junction clones and cosmid clones) (Ratnaningsih *et al.*, 1990); the other map was constructed by using two-dimensional techniques (partial/total and reciprocal gels) (Römmling *et al.*, 1989; Römmling & Tümmeler, 1991). Circularity was proven by

the use of the two enzymes *SpeI* and *DpnI* and the establishment of all links between mutually overlapping fragments.

The two maps differed in the number and assignment of some small fragments between 10 and 40 kbp, mainly SpAB, SpAD, SpAF and SpAJ (Tümmeler *et al.*, 1992). The nomenclature Sp (*SpeI*) and Dp (*DpnI*) is used to identify particular fragments, the letter indicating the fragment size (Tümmeler *et al.*, 1992). To enable a consistent consensus map, the strains used for mapping and the cosmids that led to the conflicting mapping data were exchanged between both groups and reinvestigated. Both strains revealed identical *SpeI* and *DpnI* fragment patterns: 38 *SpeI* and 15 *DpnI* fragments. In the original report by Römmling & Tümmeler (1991) the *SpeI* fragment

contig K-AC-AJ-AB-O was constructed from the pattern of linking fragments seen on two-dimensional *SpeI* partial/total gels. Additionally, it was later shown that SpAB contains a *PacI* site.

Initially, three cosmids, namely pMO010205 (hybridized to SpAF-SpAJ; cosmid contains one *SpeI* site), pMO013540 (SpAF-SpAJ-?; cosmid contains two *SpeI* sites), and pMO011505 (SpAF-SpAJ-SpQ; cosmid contains two *SpeI* sites) were used to locate SpAJ next to SpAF and SpQ (U. Römling & B. Tümmeler, unpublished data). However, subsequent Southern analysis of blots which were optimized for preferential separation of small fragments showed that pMO010205 hybridized to fragments SpAB and SpAJ/DpE, pMO013540 hybridized to fragments SpQ, SpAB and SpAJ/DpE and pMO011505 hybridized to fragments SpQ, SpAB, SpAC, SpAJ/DpE and DpB. The hybridization signals of the cosmid clones to DpE clearly placed the cosmids onto the region between the *SpeI* fragments K and H.

In the case of pMO011505, however, there was one more hybridization signal than expected. In order to try to elucidate these ambiguous hybridization data, the insert of cosmid pMO011505 was cut by *EcoRI* and subcloned into pUC8. Five different subclones (pMO1–pMO5, according to size) corresponding to the cosmid inserts were obtained and used for hybridization. pMO1 and pMO2 were revealed to be *SpeI*-linking clones which hybridized to SpAB/SpAJ and SpAC/SpAJ only. In addition pMO1 contains the expected *PacI* site. Hybridization with pMO3 and pMO4 which are non-linking clones gave strong signals with SpQ.

The anomalous hybridization behaviour of the cosmid may be explained by accidental coligation of two fragments or by a repetitive element on fragment SpAC which is more frequently located on fragment SpQ. The latter hypothesis is favoured because pMO013540 detects the same fragment, SpQ. This example shows that repetitive sequences, even when they exist in low-copy number on a bacterial genome, may lead to a wrong assignment of fragments. In contrast, two-dimensional mapping techniques proved not to be hindered by repetitive elements. Fig. 2 shows the physical consensus map of both groups and the location of genes mapped by hybridization.

Partial *DraI* (Shortridge *et al.*, 1991) and *HpaI* (Ronald *et al.*, 1992) maps are available as are *SwaI* and *PacI* maps of the PAO genome, but, for the latter, the correlations of gene location are not available (Römling & Tümmeler, 1992). Furthermore, many of the *PacI* and *SwaI* sites are closely located to existing *DpnI* and *SpeI* sites.

Concluding remarks

No matter which mapping methods are chosen, once a physical map exists it will help to elucidate problems faster and more reliably than a genetic map. The advantage of having a generally used strain of *P. aeruginosa*, strain PAO, has been considerable in assembling these data.

However, the advantage of the techniques used for the physical map is that they are readily applicable to other strains, which will be important for clinical research. As more markers are mapped, it will be possible to draw more conclusions as to the architecture of the genome and the significance of marker arrangements. The existence and origin of auxotroph-rich regions has already been discussed (Holloway & Morgan, 1986). The availability of two Hfr systems of mapping will continue to provide data on gene order on a chromosome-wide basis. Both systems use temperature-sensitive IncP-1 plasmids and are complementary in that each system provides a different set of Hfr sites (Reimmann & Haas, 1986; O'Hoy & Krishnapillai, 1987). At present the resolution of the physical map in *P. aeruginosa* PAO does not exceed the resolution of the genetic map. However, there is the technical potential to refine the scale even more, for example with cosmid mapping or transposon-mediated mapping. The data in this paper will be a valuable contribution when the finest possible resolution of the genomic structure, namely the sequencing of the *P. aeruginosa* PAO genome, is undertaken.

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