Transductional Analysis of Cysteine Mutants in *Proteus mirabilis*

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(Accepted for publication 30 September 1971)

SUMMARY

Seventy-three cysteine (*cys*) auxotrophs of *Proteus mirabilis* strain 13 were arranged in six transductional groups. Members of these groups had nutritional requirements similar to *Salmonella typhimurium* and *Escherichia coli cys* mutants. The chromosomal topography of the *P. mirabilis* mutants resembles that of *S. typhimurium* and *E. coli cys* mutants in that they are not arranged in an operon and *cysB* mutants are co-transducible with tryptophan markers. The *P. mirabilis cysG* auxotrophs may be equivalent to *S. typhimurium cysI* and *J* mutants which have similar nutritional requirements. In *P. mirabilis cysG* is closely linked to *cysC* and the *S. typhimurium cysC, D, H, I, J* cluster may also be present in this organism. The *P. mirabilis cysE* mutants, which are co-transducible with an arginine gene cluster, seem to lack *O*-acetylserine sulphydrylase activity.

INTRODUCTION

Nine cysteine (*cys*) genes have been mapped in *Salmonella typhimurium* (Fig. 2). With the exception of *cysB*, which may have a regulatory function (Spencer, Collins & Monty, 1967), the genes code for enzymes (Mizobuchi, Demerec & Gillespie, 1962; Dreyfuss & Monty, 1963; Siegel, Click & Monty, 1964; Kredich & Tomkins, 1966; Henderson & Loughlin, 1968). Apparently homologous *S. typhimurium* genes map at similar locations in *Escherichia coli* (Taylor, 1970). The *cysB-trp* linkage group represents the only known inversion of the chromosome in these organisms (Sanderson, 1970). Five transduction groups of *Pseudomonas aeruginosa cys* mutants are not linked (Calhoun & Feary, 1969) and two cysteine markers of *Bacillus subtilis* are also located far apart (Dubnau, Goldthwaite, Smith & Marmur, 1967). *Streptomyces coelicolor* resembles *S. typhimurium* in that its *cysC* and *D* genes are closely linked (Hopwood, 1967).

In *Proteus mirabilis* strain 13 (Coetzee & Sacks, 1960*a*) the cysteine pathway (Grabow & Smit, 1967) is similar to that in *Salmonella typhimurium* (Fig. 1), but the chromosomal topography of corresponding mutants has not been investigated. The *cysE* gene of *P. mirabilis* is co-transducible with the *argE, C, B, G, H* cluster (Böhme, 1964; Prozesky & Coetzee, 1966; Prozesky, 1968). These genes are also linked in *Proteus vulgaris* but not in *Providencia* (Coetzee & Prozesky, 1969).

This communication deals with transductional analysis of six phenotypic groups of *Proteus mirabilis cys* auxotrophs. The results are necessary for studies of the regulation of the cysteine pathway (Spencer *et al.* 1967) and they are of taxonomic importance (Coetzee, Smit & Prozesky, 1966; Coetzee & Prozesky, 1969; Krizsanovich, de Klerk & Smit, 1969; Jones & Sneath, 1970).

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Fig. 1. Pathway of cysteine synthesis in *Salmonella typhimurium* (Dreyfuss & Monty, 1963; Kredich *et al.* 1969). APS = adenosine 5'-phosphosulphate; PAPS = 3'-phosphoadenosine 5'-phosphosulphate; SO_4^{2-} = sulphate; SO_3^{2-} = sulphite; $\text{S}_2\text{O}_3^{2-}$ = thiosulphate; S^{2-} = sulphide; *cysA* = sulphate-thiosulphate permease; *cysB* = gene concerned with adenylyl sulphate-3'-phosphate reductase, sulphite reductase and thiosulphate reductase; *cysC* = APS kinase; *cysD* = ATP sulphurylase; *cysE* = serine transacetylase activity of the cysteine synthetase enzyme; *cysG*, *I*, *J* = genes concerned with sulphite reductase; *cysH* = adenylyl sulphate-3'-phosphate reductase.

METHODS

Media and chemicals. The minimal medium (MM) agar has been described (Grabow & Smit, 1967) and the broth was that of Coetzee & Sacks (1960b). *O*-Acetyl-DL-serine was obtained from Dr Theodor Schuchardt, München, Germany; vitamin B_{12} from British Drug Houses, Poole, Dorset; *S*-methyl-L-cysteine from Calbiochem, California, U.S.A.; DL-cystathionine from Sigma Chemical Company, St Louis, U.S.A.; and all other amino acid growth factors from Nutritional Biochemical Corporation, Cleveland, Ohio, U.S.A. Inorganic sources of sulphur were analytical grade products (Postgate, 1963) from E. Merck Darmstadt, Germany. *O*-Succinyl-DL-homoserine was prepared according to the method of Flavin & Slaughter (1965).

Bacteria. Cysteine auxotrophs were obtained by exposing *Proteus mirabilis* strain 13 to ultraviolet light or manganese chloride+hydrogen peroxide (Grabow & Smit, 1967). Nutritional requirements were determined auxanographically (Grabow & Smit, 1967) and by streaking on MM agar containing growth factors (Mizobuchi *et al.* 1962). For the latter test MM agar was supplemented with sulphur-containing substances at concentrations of 2×10^{-2} M (molar with respect to sulphur). Additional tests were done with thiosulphate at a concentration of 2×10^{-4} M (Mizobuchi *et al.* 1962). Glycine, serine and homoserine were incorporated at $50 \mu\text{g/ml}$ and vitamin B_{12} at $0.1 \mu\text{g/ml}$ (Grabow, 1972). Results were recorded after 48 h incubation. Growth was regarded as positive when it was comparable to that of the mutant on cysteine-supplemented MM agar. Double mutants were isolated by the methods of Prozesky & Coetzee (1966) and Prozesky (1967). Arginine mutants were kindly supplied by Professor O. W. Prozesky and tryptophan markers were isolated and characterized by the above methods. Strains were maintained on agar slopes at 4° . Subcultures were made regularly to prevent the selection of double mutants (Gillespie, Demerec & Itikawa, 1968). Incubation was at 37° .

Transduction. The general phage techniques were those of Adams (1959). Lysates of the transducing phage 34.13 (Coetzee & Sacks, 1960a) with plaque-forming titres of 5×10^8 to $2 \times 10^{10}/\text{ml}$ were prepared by an agar layer technique (Prozesky, de Klerk & Coetzee, 1965). Final lysates contained less than 50 plaque-forming units of phage 13 *vir* per ml (Prozesky, 1968; Coetzee & Smit, 1970). The transduction procedure has been described

(Prozesky & Coetzee, 1966; Prozesky, 1968). Controls for absence of viable bacteria in phage lysates as well as controls in which recipients were treated with lysates prepared on homologous and wild-type bacteria respectively were included. Wild-type transductants were selected on MM agar and donor-type transductants on MM agar supplemented with appropriate growth factors. Plates were scored after 48 h and those with donor-type transductants replicated to MM agar for detection (Lederberg & Lederberg, 1952). Purity of transductant clones was tested according to Prozesky (1968).

Genetic mapping. Co-transduction of genes was tested by two-point crosses, donor-phenotype transductions and transduction of double mutants with wild-type donors (Demerec *et al.* 1956; Clowes, 1958*b*; Mizobuchi *et al.* 1962; Prozesky & Coetzee, 1966; Prozesky, 1968).

RESULTS

Auxotrophic mutants. Seventy-three non-leaky cysteine mutants of *Proteus mirabilis* strain 13 with low reversion frequencies were divided into six phenotypic groups by their growth responses to precursors of cysteine (Table 1).

The nutritional requirements of the *cysA*, *B*, *C*, *K* and *G* groups resemble those of *cysA*, *B*, *C*, *E* and *G* mutants of *Salmonella typhimurium* (Clowes, 1958*a*; Mizobuchi, *et al.* 1962). Mutants with nutritional requirements similar to those of the *Proteus mirabilis cysE* auxotrophs have not been described in *S. typhimurium* or *Escherichia coli*. None of the auxotrophs responded to dithionate, sulphur, lanthionine, cysteic acid, glycine, serine, homoserine, *O*-succinylhomoserine or vitamin B₁₂. Growth response to cysteine sulphinic acid was not tested since it serves as a source of sulphite (Leinweber & Monty, 1961) and yields results similar to metabisulphite and sulphite in *S. typhimurium* (Mizobuchi *et al.* 1962). Differences between *P. mirabilis* mutants and the analogous *S. typhimurium* auxotrophs are that the *P. mirabilis* strains do not respond to lanthionine (Demerec *et al.* 1956; Clowes, 1958*a*) and that *cysA* mutants do not grow with thiosulphate (Mizobuchi *et al.* 1962). The *P. mirabilis cysA*, *C*, *E* and *G* mutants grow equally well on cysteine or methionine whereas homologous *E. coli* (Lampen, Jones & Perkins, 1947; Simmonds, 1948) and *S. typhimurium* (Demerec *et al.* 1956; Clowes, 1958*a*) auxotrophs grow poorly on methionine. In the latter respect the *P. mirabilis* mutants resemble similar auxotrophs of *Neurospora* (Horowitz, 1947) and *Pseudomonas aeruginosa* (Calhoun & Feary, 1969). The inability of *cysB* and *K*

Table 1. *Nutritional requirements of Proteus mirabilis cysteine auxotrophs*

Growth factor	Growth response of mutants					
	<i>cysA</i>	<i>cysC</i>	<i>cysG</i>	<i>cysB</i>	<i>cysK</i>	<i>cysE</i>
Sulphate	—	—	—	—	—	—
Dithionite	+	+	+	—	—	—
Metabisulphite	+	+	—	—	—	—
Sulphite	+	+	—	—	—	—
Thiosulphate	—	+	+	—	—	—
Sulphide	+	+	+	+	—	—
Cysteine	+	+	+	+	+	+
<i>S</i> -Methylcysteine	+	+	+	—	—	+
<i>O</i> -Acetylserine	—	—	—	—	+	—
Cystathionine	+	+	+	—	—	+
Homocysteine	+	+	+	—	—	+
Methionine	+	+	+	—	—	+
Number of mutants in each group	9	5	30	3	8	18

mutants to grow with *S*-methylcysteine, cystanthionine, homocysteine or methionine cannot be explained. The *P. mirabilis* *cysB* mutants seem to resemble members of the *S. typhimurium* *cysBb* complementation group which do not respond to thiosulphate or dithionite (Mizobuchi *et al.* 1962). Cultures of cysteine mutants were kept on agar slopes at 4° for more than a year and double mutants (Gillespie *et al.* 1968) did not appear.

All the mutants used behaved like point mutations. No true deletions (Hartman, Loper & Šerman, 1960) or mixed transductant clones (Prozesky, 1968) were encountered. Ditto deletions of *cys* genes like those in *Salmonella typhimurium* (Itikawa & Demerec, 1967) were not detected. *Proteus mirabilis* seems to resemble *Escherichia coli* in this respect (Jones-Mortimer, 1968).

Table 2. Two-point transductions of *cys* mutants

Recipient	Donor					
	<i>cysA1</i>	<i>cysA2</i>	<i>cysB14</i>	<i>cysB20</i>	<i>cysC1</i>	<i>cysC3</i>
<i>cysA1</i>	0	5	1066	1183	1202	1136
<i>cysA2</i>	3	0	1126	1281	1242	1202
<i>cysA354</i>	2	4	1253	1186	1293	1293
<i>cysB14</i>	1138	1147	0	2	1125	1193
<i>cysB17</i>	1242	1183	1	2	1021	987
<i>cysB20</i>	1123	1202	2	0	1034	1086
<i>cysC1</i>	939	901	862	894	0	0
<i>cysC3</i>	967	875	897	893	1	0
<i>cysC666</i>	983	976	945	947	2	1
<i>cysE6</i>	1136	1286	1223	1123	1208	1242
<i>cysE24</i>	1220	1291	1231	1109	1070	989
<i>cysE448</i>	1219	1287	1262	1207	1139	1252
<i>cysG2</i>	795	838	828	818	464	538
<i>cysG20</i>	789	894	786	874	421	636
<i>cysG1795</i>	939	875	847	963	434	674
<i>cysK13</i>	880	921	821	818	763	649
<i>cysK22</i>	842	901	827	874	765	794
<i>cysK32</i>	837	985	898	939	871	861
<i>argE1</i>	364	401	276	331	287	322
<i>trp-1</i>	2289	2386	2124	2112	2217	2340
	<i>cysE6</i>	<i>cysE24</i>	<i>cysG2</i>	<i>cysG20</i>	<i>cysK13</i>	<i>I3</i>
<i>cysA1</i>	1083	1145	1124	1030	1268	1127
<i>cysA2</i>	1249	1206	1287	1206	1010	1208
<i>cysA354</i>	1307	1286	1176	1147	1374	1284
<i>cysB14</i>	1134	1089	1090	1186	1248	1186
<i>cysB17</i>	1143	1124	981	975	2020	1080
<i>cysB20</i>	1243	1206	1034	1081	2031	1123
<i>cysC1</i>	949	913	565	636	989	921
<i>cysC3</i>	868	973	574	706	1038	946
<i>cysC666</i>	1083	969	650	638	1092	1021
<i>cysE6</i>	0	1	1286	1202	1108	1212
<i>cysE24</i>	3	0	1135	1143	1021	1099
<i>cysE448</i>	4	3	1180	1247	1295	1235
<i>cysG2</i>	891	842	0	1	868	870
<i>cysG20</i>	935	946	0	1	825	913
<i>cysG1795</i>	986	974	3	2	881	942
<i>cysK13</i>	798	869	873	626	0	808
<i>cysK22</i>	846	823	842	813	4	842
<i>cysK32</i>	891	934	808	747	6	873
<i>argE1</i>	286	231	342	312	325	308
<i>trp-1</i>	2281	2310	2396	2286	2321	2342

Selection was for wild-type. Colony numbers on control plates were subtracted.

Linked transduction of cysteine genes. Reciprocal two-factor crosses yielded relatively low transduction frequencies between *cysC* and *G* and between *cysE* and *argE* mutants which indicates that these pairs are cotransducible (Table 2).

Small numbers of transductants were obtained from crosses between mutants which belonged to the same phenotypic group which indicated that the six phenotypic groups (Table 1) may be equivalent to different genes (Demerec *et al.* 1956). It does not exclude the possibility that some mutants with similar nutritional requirements may represent two or more closely linked genes (Mizobuchi *et al.* 1962). Numbers of colonies in control crosses between mutants and homologous donors were low (Table 2) which showed that the rever-

Table 3. *Donor-phenotype transductions of cysG mutants with a cysC donor*

Cross		Transductants Prototrophs/total	Percentage linkage
Recipient	Donor		
<i>cysG9</i>	× <i>cysCI</i>	67/671	90.01
<i>cysG11</i>	× <i>cysCI</i>	69/678	98.82
<i>cysG15</i>	× <i>cysCI</i>	82/788	89.59
<i>cysG1269</i>	× <i>cysCI</i>	92/882	89.57
<i>cysG6</i>	× <i>cysCI</i>	85/810	89.51
<i>cysG306</i>	× <i>cysCI</i>	103/974	89.43
<i>cysG5</i>	× <i>cysCI</i>	75/704	89.35
<i>cysG3</i>	× <i>cysCI</i>	84/780	89.23
<i>cysG8</i>	× <i>cysCI</i>	98/907	89.20
<i>cysG1262</i>	× <i>cysCI</i>	91/837	89.13
<i>cysG7</i>	× <i>cysCI</i>	82/840	88.92
<i>cysG14</i>	× <i>cysCI</i>	105/940	88.83
<i>cysG17</i>	× <i>cysCI</i>	84/741	88.66
<i>cysG373</i>	× <i>cysCI</i>	87/750	88.40
<i>cysG20</i>	× <i>cysCI</i>	93/771	87.94
<i>cysG1</i>	× <i>cysCI</i>	93/762	87.80
<i>cysG2</i>	× <i>cysCI</i>	81/635	87.24
<i>cysG4</i>	× <i>cysCI</i>	106/809	86.90
<i>cysG19</i>	× <i>cysCI</i>	111/815	86.38
<i>cysG1795</i>	× <i>cysCI</i>	96/666	85.59
<i>cysG16</i>	× <i>cysCI</i>	106/706	84.99

Table 4. *Reciprocal donor-phenotype transductions between cysE mutants and argE1*

Cross		Transductants Prototrophs/total	Percentage linkage	Average
Recipient	Donor			
<i>cysE7</i>	× <i>argE1</i>	520/682	23.75	23.18
<i>argE1</i>	× <i>cysE7</i>	421/544	22.61	
<i>cysE663</i>	× <i>argE1</i>	472/614	23.13	23.17
<i>argE1</i>	× <i>cysE663</i>	533/694	23.20	
<i>cysE699</i>	× <i>argE1</i>	389/507	23.27	22.86
<i>argE1</i>	× <i>cysE699</i>	463/597	22.45	
<i>cysE9</i>	× <i>argE1</i>	524/688	23.84	22.75
<i>argE1</i>	× <i>cysE9</i>	539/688	21.66	
<i>cysE30</i>	× <i>argE1</i>	463/600	22.83	22.45
<i>argE1</i>	× <i>cysE30</i>	558/716	22.07	
<i>cysE6</i>	× <i>argE1</i>	337/435	22.53	22.14
<i>argE1</i>	× <i>cysE6</i>	385/492	21.75	
<i>cysE554</i>	× <i>argE1</i>	426/549	22.40	21.78
<i>argE1</i>	× <i>cysE554</i>	481/610	21.15	
<i>cysE1002</i>	× <i>argE1</i>	521/671	22.35	21.88
<i>argE1</i>	× <i>cysE1002</i>	426/542	21.40	

sion frequency of the mutants was low. Linkage between *cysB* and *trp* is probably not detectable in two-point crosses since the genes are located far apart. Co-transduction of *cysC* and *G*, *cysE* and *argE*, and *cysB* and *trp* was demonstrated in donor-phenotype transductions (Tables 3, 4 and 5, respectively) and by transduction of double mutants to prototrophy with wild-type donors. Reciprocal crosses between *cysC* and *G* (Table 3) were not possible since *cysG* represents a block distal to *cysC* and the *cysG* phenotype cannot be selected in crosses with *cysG* donors. The average percentage linkage values calculated from the donor-phenotype transductions are 88.8 % for *cysC-G*, 22.5 % for *cysE-argE* and 1.5 % for *cysB-trp*.

DISCUSSION

The nutritional requirements of the six phenotypic groups of cysteine auxotrophs confirm findings (Grabow & Smit, 1967) that the pathways of synthesis of this amino acid are similar in *Proteus mirabilis*, *Salmonella typhimurium* and *Escherichia coli* (Dreyfuss & Monty, 1963; Jones-Mortimer, 1968; Becker & Tomkins, 1969). This pathway seems to operate also in *Pseudomonas aeruginosa* (Calhoun & Feary, 1969). Growth responses to *O*-acetylserine indicate that *P. mirabilis cysK* auxotrophs resemble *cysE* mutants of *S. typhimurium* and *E. coli* which are deficient in the serine transacetylase activity of the cysteine synthetase enzyme (Jones-Mortimer, Wheldrake & Pasternak, 1968). The *P. mirabilis cysE* mutants seem to lack the *O*-acetylserine sulphydrylase activity of the latter enzyme, a phenotype which despite various efforts (Becker & Tomkins, 1969; Kredich, Becker & Tomkins, 1969) has not been detected amongst *S. typhimurium* or *E. coli* mutants. It thus appears that *P. mirabilis cysE* auxotrophs are not the equivalent of *cysE* mutants of *S. typhimurium* as has previously been reported (Prozesky & Coetzee, 1966; Grabow & Smit, 1967; Prozesky, 1968).

The scattered arrangement of cysteine genes and co-transduction of *cysB* and *trp* in *Proteus mirabilis* has also been reported in *Salmonella typhimurium* (Mizobuchi *et al.* 1962) and *Escherichia coli* (Jones-Mortimer, 1968). The *P. mirabilis cysG* auxotrophs, which are closely linked to *cysC*, have nutritional requirements similar to *cysG*, *I* or *J* mutants of *S. typhimurium* and it seems that the *cysC*, *D*, *H*, *I*, *J* cluster of the latter organism is also present in *P. mirabilis*. It would appear that the topography of cysteine genes in *P. mirabilis* resembles that of analogous genes in *S. typhimurium* (Fig. 2) and *E. coli*. Experiments on the isolation of a marker which can be used to determine whether the orientation of the *cysB-trp* linkage group in *P. mirabilis* corresponds to that in *S. typhimurium* or *E. coli* are in progress. Similarities in topography between *P. mirabilis* and *S. typhimurium* or *E. coli* have also been found with arginine (Prozesky, 1968) and methionine genes (Grabow, 1972).

The distance between *cysB* and *trp* seems to be different in *Proteus mirabilis* and *Sal-*

Table 5. Reciprocal donor-phenotype transductions between *cysB* mutants and *trp-1*

Cross		Transductants Prototrophs/total	Percentage linkage	Average
Recipient	Donor			
<i>cysB14</i>	× <i>trp-1</i>	562/568	1.1	1.0
<i>trp-1</i>	× <i>cysB14</i>	631/636	0.8	
<i>cysB20</i>	× <i>trp-1</i>	625/636	1.7	1.5
<i>trp-1</i>	× <i>cysB20</i>	734/744	1.3	
<i>cysB17</i>	× <i>trp-1</i>	538/548	1.8	1.9
<i>trp-1</i>	× <i>cysB17</i>	593/605	2.0

Fig. 2. Topography of cysteine genes in *Salmonella typhimurium* (Mizobuchi *et al.* 1962; Sanderson, 1970). Genes are shown on the outside of the circle. Arabic numbers on the inside indicate interrupted conjugation time intervals in min. Linked genes appear on the same line in clockwise order from left to right.

monella typhimurium since the percentage linkage between these markers is 1.5 % in the former (Table 5) and 56 to 82 % in the latter (Demerec & Hartman, 1956). The difference in these values reflects a difference in chromosomal distance since the transducing phages 13 and P22 have similar head sizes (Prozesky *et al.* 1965) and are thus expected to carry equivalent sections of host chromosome (Lennox, 1955; Enomoto, 1967; Sanderson, 1970; Mise & Suzuki, 1970). Genetic differences between *P. mirabilis* and *S. typhimurium* are expected since they have different guanine + cytosine molar contents (see Grabow, 1972).

This work was partly supported by grants from the South African Medical Research Council to Professor J. N. Coetzee.

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