TRANSDUCTIONAL ANALYSIS OF METHIONINE GENES IN *PROTEUS MIRABILIS*

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1. Introduction

Six structural genes (Smith, 1961; Smith and Childs, 1966; Childs and Smith, 1969) and two regulatory genes (Ayling and Chater, 1968; Lawrence, Smith and Rowbury, 1968; Chater and Rowbury, 1970) of the methionine pathway have been mapped in *Salmonella typhimurium* (see figs. 1 and 2). The requirement for methionine of another group of *S. typhimurium* mutants is due to an altered methionyl-sRNA synthetase (Gross and Rowbury, 1971). Loci homologous to some of the *S. typhimurium* genes have been identified in *Escherichia coli* and they map at corresponding locations (see Taylor, 1970). Apart from the *metA*, *H*, and *metJ*, *B*, *F* clusters the members of which are not involved in consecutive biosynthetic steps, the methionine genes of *S. typhimurium* are scattered over the genome (Smith, 1961; Ayling and Chater, 1968). Four methionine structural genes of *Pseudomonas aeruginosa* (Calhoun and Feary, 1969) are also not arranged in an operon (Jacob and Monod, 1961). In contrast three methionine structural genes of *Staphylococcus aureus* are linked together in an " assembly line " (Harmon *et al.*, 1966).

Cooper (1966) identified an *E. coli* gene which is involved in the conversion of D-methionine to the L-isomer. Methionine auxotrophs with a mutation in this gene do not respond to D-methionine whereas the wild-type grows equally well with D- or L-methionine (Lampen, Jones and Perkins, 1947; Huang, 1963) under aerobic conditions (Cooper, 1966). In interrupted mating experiments this gene was located near the *lac* region (Cooper, 1966) far from other methionine genes (see Taylor, 1970).

In *Proteus mirabilis* strain 13 the methionine pathway is similar to that in *S. typhimurium* and *E. coli* (Grabow and Smit, 1967) but the location of the genes was unknown. *P. mirabilis* is different in that it may synthesise methionine *via* S-methylcysteine (SMC—Grabow and Smit, 1967). This paper concerns the isolation and characterisation of *P. mirabilis* methionine structural and regulatory genes and a gene involved in the conversion of D- to L-methionine. Linkage studies by transduction provide information which relates to the regulation of methionine synthesis (Lawrence *et al.*, 1968; Childs and Smith, 1969), the relevance of SMC, and is of taxonomic interest (Coetzee, Smit and Prozesky, 1966; Jones and Sneath, 1970). To permit comparisons between linkage maps the nomenclature is the same as that used for corresponding *S. typhimurium* and *E. coli* genes (see Sanderson, 1970; Taylor, 1970).

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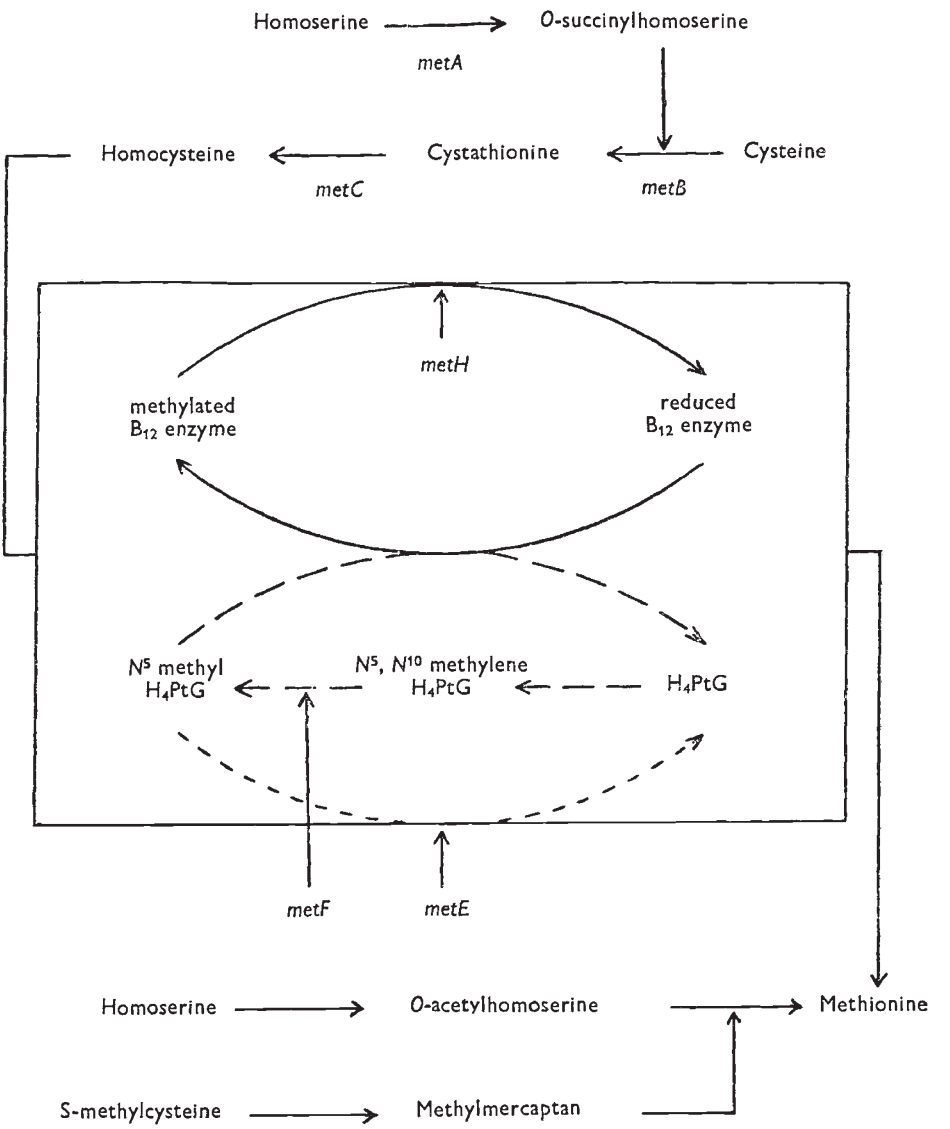

FIG. 1.—The pathway of methionine synthesis in *Salmonella typhimurium* (after Smith and Childs, 1966; Childs and Smith, 1969) and in *Neurospora via* S-methylcysteine (after Moore and Thompson, 1967). Short broken line arrow = tetrahydropteroyl-triglutamate only; long broken line arrow = either tetrahydropteroyltri- or monoglutamate; *metA* = homoserine *O*-transsuccinylase; *metB* = cystathionine synthetase; *metC* = cystathionase; *metE* = N^5-methyltetrahydropteroyl-triglutamate-homocysteine transmethylase; *metF* = N^5, N^{10}-methylene tetrahydrofolate reductase; *metH* = cobalamin-dependent N^5-methyltetrahydrofolate-homocysteine transmethylase.

2. MATERIALS AND METHODS

Media and chemicals

The minimal medium (MM) agar was that of Grabow and Smit (1967) and the broth that of Coetzee and Sacks (1960*a*). D- and L- isomers of

methionine and DL-ethionine were obtained from Dr Theodor Schuchardt, München, Germany; vitamin B_{12} and DL-norleucine from British Drug Houses, Poole, England; DL-cystathionine from Sigma Chemical Company, St Louis, U.S.A.; *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) from Aldrich Chemical Co., Milwaukee, U.S.A., and S-methyl-L-cysteine (SMC) from Calbiochem, California, U.S.A. All other growth factors were supplied by Nutritional Biochemicals Corporation, Cleveland, U.S.A., and *O*-succinyl-DL-homoserine was prepared by the method of Flavin and Slaughter (1965).

Bacteria

Mutants were derived from wild-type *Proteus mirabilis* strain 13 or its streptomycin-resistant mutant *str-r1* (13 *str-r* of Coetzee and Sacks, 1960*b*). Auxotrophs were obtained after induction with ultraviolet light, manganese chloride or hydrogen peroxide (Grabow and Smit, 1967) or NG using the methods of Adelberg, Mandel and Chein Ching Chen (1965) or Childs and Smith (1969). Mutants were characterised auxanographically (Grabow and Smit, 1967). Strain *metE3* (table 1) was used to isolate mutants of the *metEmetH* genotype as described by Childs and Smith (1969) and *metE3* mutants with leaky secondary mutations were discarded as they could have been *metE3metG* mutants (Smith and Childs, 1966). Eight phenotypically different arginine auxotrophs of *P. mirabilis* strain 13 were kindly provided by Professor O. W. Prozesky (Prozesky, 1967, 1968).

Using the methods of Lawrence *et al.* (1968) mutants resistant to inhibition by the methionine analogues ethionine and norleucine (1 mg./ml.) were isolated and their excretion of methionine tested by syntrophism tests at 25° C. or 37° C. in which the mutants were streaked parallel to *metA* or *metF* auxotrophs on MM agar. Attempts to isolate methionine auxotrophs deficient in D-methionine utilisation were in accordance with Cooper (1966) except that NG was used for mutagenesis instead of ultraviolet light. Double auxotrophs were isolated as described by Prozesky and Coetzee (1966) and Prozesky (1967), and auxotrophs resistant to inhibition by methionine analogues according to Ayling and Chater (1968). The final group of mutants used consisted of one mutant of each phenotype retained from each isolation experiment. All strains were kept on agar slopes at 4° C. and subcultured once every 6 months. Each subculture was routinely tested for reversions. Unless otherwise stated, incubation was at 37° C.

Transduction

Phage preparation and assay techniques were those of Adams (1959). Lysates of the transducing phage 34·13 (Coetzee and Sacks, 1960*a*) containing 5×10^9-2×10^{10} plaque-forming units/ml. were prepared by an agar-layer technique (Prozesky, de Klerk and Coetzee, 1965). Final lysates contained less than 50 plaque-forming units/ml. 13*vir* (Prozesky, 1968; Coetzee and Smit, 1970). The transduction procedure of Prozesky and Coetzee (1966) and Prozesky (1968) was used. Controls to detect bacteria in phage preparations and those in which recipients were treated with lysates prepared on homologous and wild-type bacteria respectively were included. All plates were incubated for 48 hours.

Wild-type transductants were selected on MM agar and auxotrophic donor phenotype transductants on MM agar + 50 μg./ml. of the required growth factor (Smith and Childs, 1966). *MetE* mutants were grown on

MM agar + 0·1 μg./ml. vitamin B_{12} (Smith, 1961). Wild and donor-type transductants were distinguished after replication to MM agar and incubation for 24 hours (Lederberg and Lederberg, 1952). The methods of Ayling and Chater (1968) were used to select for resistance to inhibition by methionine analogues and the selection of markers linked to *metH* was as described by Childs and Smith (1969).

Genetical mapping

Linkage was tested by the frequency of co-transduction in two-point crosses, donor-phenotype transduction and the transduction of double mutants with phage prepared on the wild-type (Smith, 1961; Ayling and Chater, 1968; Prozesky, 1968; Childs and Smith, 1969).

3. Results

(i) *Auxotrophs*

The growth responses of 55 single-site auxotrophs (table 1) to intermediates in the methionine pathway indicate that they represent mutations at three different gene loci similar to those of the *metA*, *E* and *F* mutants of *Salmonella typhimurium* (Smith and Childs, 1966) and so they were designated accordingly. Auxotrophs similar to *metB*, *C* and *G* mutants of *S. typhimurium* (Smith and Childs, 1966) were not encountered. On the basis of previous

Table 1

Growth responses of methionine auxotrophs of Proteus mirabilis *to methionine intermediates*

	Growth responses of mutant groups			
Intermediate	*metA*	*metE*	*metF*	*metEmetH*
Cysteine	−	−	−	−
Homoserine	−	−	−	−
O-succinylhomoserine	+	−	−	−
Cystathionine	+	−	−	−
Homocysteine	+	−	−	−
Vitamin B_{12}	−	+	−	−
S-methylcysteine	−	+	+	+
Methionine	+	+	+	+
Number of mutants in each group	16	28	11	21

studies of the metabolic defects of some of these mutants (Grabow and Smit, 1967) the ability of *metE* and *F*, and the inability of *metA* auxotrophs to utilise SMC is shared with similar auxotrophs of *Neurospora crassa* (Wiebers and Garner, 1964; Moore and Thompson, 1967) but differs from those of *Escherichia coli* (Grabow and Smit, 1967) and *Pseudomonas aeruginosa* (Calhoun and Feary, 1969).

Treatment of *metE3* cultures with NG yielded mutants (table 1) which retained the ability to grow with methionine but no longer respond to vitamin B_{12}. These auxotrophs probably possess the same defects as *metEmetH* mutants of *S. typhimurium* (Childs and Smith, 1969) for which genetical evidence will be given later.

The phenotypes of the *metE* and *metEmetH* mutants indicate that the two routes for the methylation of homocysteine catalysed by the N^5-methyl-

tetrahydropteroyltriglutamate-homocysteine transmethylase (*metE*) and cobalamin-dependent N^5-methyltetrahydrofolate-homocysteine transmethylase (*metH*) enzymes described for *S. typhimurium* (Woods, Foster and Guest, 1965; Childs and Smith, 1969) also occur in *P. mirabilis* and that like *S. typhimurium* (Cauthen, Foster and Woods, 1966) and *E. coli* (Galivan and Huennekens, 1970) the intracellular concentration of vitamin B_{12} is low in *P. mirabilis* so that the *metH* route only functions when vitamin B_{12} is supplied. As homocysteine is normally methylated *via* the *metE* route, *metH* mutants are phenotypically wild and *metH* mutations can only be identified in *metE* auxotrophs (see Childs and Smith, 1969).

(ii) *Mutants resistant to inhibition by methionine analogues*

Two groups of resistant mutants were isolated, one resistant to ethionine and the other resistant to both ethionine and norleucine. Eight members of the latter group were isolated on MM agar + norleucine, and the remaining three members of that group and the five members of the former group on MM agar + ethionine. Colony growth of all the mutants on MM agar was slow as compared with wild-type. The similarity to the *metJ* and *K* regulatory mutants of *S. typhimurium* (Lawrence *et al.*, 1968) justified use of the same designation. The *P. mirabilis* mutants differed from the *S. typhimurium* mutants in that they appeared not to overproduce methionine at either 37° C. or 25° C. As no α-DL-methylmethionine was available it was not possible to isolate mutants like the *metI* mutants of *S. typhimurium* (Lawrence *et al.*, 1968).

(iii) *Methionine auxotrophs unable to utilise D-methionine*

All methionine auxotrophs of *P. mirabilis* grew equally well on MM agar + either D- or L-methionine, similar to the situation in *E. coli* (Cooper, 1966). Cultures of *metF1*, *F5*, *E4*, *E5*, *A2*, and *A3* were treated with NG and about 1×10^5 clones of each were screened by replica plating for inability to grow on D-methionine. No such mutants were encountered which is surprising since Cooper isolated many of them from *E. coli*.

(iv) *Transductional analysis of* met *mutants*

Reciprocal two-point crosses in all combinations between *metA*, *E* and *F* mutants yielded less than 12 wild-type transductants in crosses between mutants of the same phenotype whereas more than 1000 were obtained in crosses with different phenotypes (table 2). The numbers of prototrophs in the latter crosses were comparable to those in crosses with the wild-type donor. These results indicate that the *metA*, *E* and *F* auxotrophs represent three genes which are not co-transducible (see Smith, 1961). In crosses with *metA* recipients the yield of wild-type recombinants was always more than 4000 which is about twice as high as that with *metE* or *F* recipients (table 2). The donor capacity of *metA* mutants did not differ from that of the latter two groups. *MetE855* and *metE933* had poor recipient and donor capacities and are possibly multisite mutants (Demerec and Ozeki, 1959; Hartman, Loper and Šerman, 1960). *MetE572* and *metE291* were poor recipients but were normal as donors; they may be deficient in recombination and replication of deoxyribonucleic acid (Alberts and Frey, 1970). The results of crosses between *metEmetH* mutants plated on MM agar + vitamin B_{12} followed by replication to MM agar to detect *metEmetH*$^+$ recombinants, indicated (table 3) that all

metH mutations are located close together, perhaps in one gene (Childs and Smith, 1969).

The methionine mutants behaved like point mutations since they yielded revertants at some stage of the investigation and they could participate as donors or recipients in transduction experiments. None of the mutants had the characteristics of deletion mutations (Hartman *et al.*, 1960; Itikawa and Demerec, 1967). No mixed colonies were encountered among transductant clones.

TABLE 2

The frequency of transduction between met *auxotrophs*

	Donor						
Recipient	*metA1*	*metA2*	*metE1*	*metE3*	*metF1*	*metF2*	wild-type
metA1	0	11	4840	4620	4740	4980	4740
metA2	1	2	4730	4540	4600	4890	4640
metA1303	3	8	5342	5110	5280	5340	5230
metE1	1620	1634	0	3	1584	1542	1602
metE3	1586	1620	2	1	1434	1590	1597
metE687	1573	1640	6	4	1740	1620	1638
metF1	2342	2394	2180	2420	0	7	2383
metF2	2367	2247	2347	2407	6	0	2336
metF1445	2212	2200	2208	2286	8	3	2217

Wild-type recombinants were selected and colony numbers on control plates subtracted.

TABLE 3

The frequency of transduction between metE3metH *mutants*

	Donor					
Recipient	*metE3 metH1*	*metE3 metH3*	*metE3 metH10*	*metE3 metH12*	*metE3 metH20*	wild-type
metE3metH1	0	0	4	3	9	986
metE3metH3	3	1	4	8	4	932
metE3metH10	6	2	0	10	2	1021
metE3metH12	8	0	7	0	5	922
metE3metH20	2	1	6	11	0	956

Selection was for *metEmetH*$^+$ recombinants.

Donor-phenotype transduction was detected only between *metA* and *H* mutants. Typical results of crosses in which *metE3metH* mutants were transduced with *metA* donors appear in table 4. The counts of *metEmetH*$^+$ recombinants in table 4 were obtained by dividing the number of colonies on MM agar + vitamin B_{12} by two since a preliminary test indicated that the ratio of the relative numbers of *metE*$^+$*metH* and *metEmetH*$^+$ recombinants in the latter crosses did not differ significantly from 1 : 1 (see Lawrence *et al.*, 1968; Childs and Smith, 1969). Reciprocal crosses were not possible since *metA* represents a block proximal to *metH* in methionine synthesis (see fig. 1) and selection of donor-phenotype transductants is not possible. Organisms with the three mutations *metE, H* and *A* could have been selected and used to prove linkage between *metA* and *H* by transduction with the wild-type donor

but this was not attempted. It is also not possible to construct these mutants by transduction (Glansdorff, 1965) since phage 34·13 causes lysogenic conversion (Coetzee, 1961) which results in non-adsorption of homologous phage (see Prozesky, 1968).

TABLE 4

Donor-phenotype transduction in crosses between metEmetH *and* metA *mutants*

Cross		Transductants*	Percentage
Recipient	Donor	Prototrophs/total	linkage
metE3metH1	× *metA1*	338/514	34·2
metE3metH3	× *metA2*	462/682	32·3
metE3metH8	× *metA3*	424/649	34·7
metE3metH12	× *metA4*	521/785	33·6
metE3metH19	× *metA5*	473/708	33·2
metE3metH4	× *metA6*	444/661	32·8
metE3metH5	× *metA253*	395/589	32·9
metE3metH9	× *metA1303*	363/543	33·2
metE3metH10	× *metA1599*	478/721	33·7
metE3metH13	× *metA1*	422/628	32·8

* Selection was on MM agar+vitamin B_{12}+homocysteine and replication was to MM agar+vitamin B_{12}. Correction was made for *metE*$^+$ *metH* recombinants which are phenotypically wild (see text).

Co-transduction was not detected between methionine and arginine mutants.

4. DISCUSSION

The nutritional requirements of the *metA*, *E* and *F* auxotrophs indicate that the methione pathways (fig. 1) of *S. typhimurium* (Smith and Childs, 1966), *E. coli* (Balish and Shapiro, 1966), *St. aureus* (Harmon *et al.*, 1966) and *Ps. aeruginosa* (Calhoun and Feary, 1969) also function in *P. mirabilis*. *P. mirabilis* methylates homocysteine normally *via* the *metE* (N^5-methyltetrahydropteroyltriglutamate-homocysteine transmethylase) route and *via metH* (cobalamin-dependent N^5-methyltetrahydrofolate-homocysteine transmethylase) only when supplied with vitamin B_{12} as described for *E. coli* and *S. typhimurium* (Childs and Smith, 1969). In *Aerobacter aerogenes* both routes function without a requirement for vitamin B_{12} (Morningstar and Kisliuk, 1965) but in *Ps. denitrificans* (Lago and Demain, 1969) and in mammalian cells (Mangum and North, 1968) only the *metH* route functions. Fungi and higher plants (Lago and Demain, 1969) and apparently also *Ps. aeruginosa* (Calhoun and Feary, 1969) synthesise methionine exclusively *via* the *metE* route.

The growth response of *metE* and *F* auxotrophs to SMC indicates that *P. mirabilis* synthesises methionine *via* an alternate route also present in *Neurospora* (Wiebers and Garner, 1964) but absent in *E. coli* (Grabow and Smit, 1967) and *Ps. aeruginosa* (Calhoun and Feary, 1969). The phenotypical similarity between *metA* auxotrophs of *P. mirabilis* and the *me-5* mutant of *Neurospora* is in agreement with methionine synthesis *via* SMC (fig. 1) in *P. mirabilis* since *me-5* has a block in the SMC route (Moore and Thompson, 1967). Methionine synthesis in *P. mirabilis* may be regulated like that in

S. typhimurium since the *metJ* and *K* mutants of *P. mirabilis* are phenotypically similar to the regulatory mutants of *S. typhimurium* (Lawrence *et al.*, 1968). Overproduction of methione by the *metJ* and *K* mutants of *P. mirabilis* was possibly not detected because of insensitivity of the syntrophism test used (Lawrence *et al.*, 1968). Although mutants deficient in the utilisation of D-methionine were not isolated, the finding that methionineless *P. mirabilis* auxotrophs grow equally well with both isomers of methionine indicates that the conversion of D- to L-methionine *via* keto-methionine operative in *E. coli* (Cooper, 1966) may also occur in *P. mirabilis*.

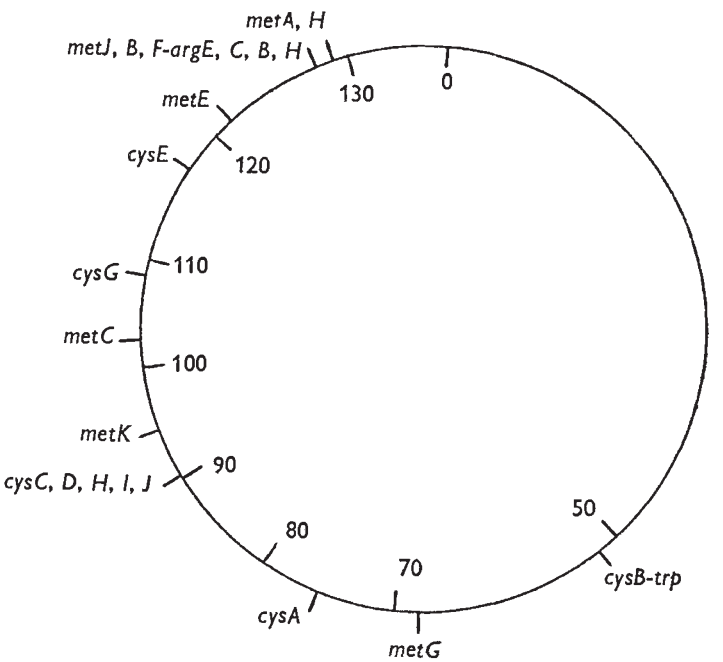

FIG. 2.—Topography of methionine genes in *Salmonella typhimurium* (after Ayling and Chater, 1968; Sanderson, 1970). Genes are shown on the outside of the circle; arabic numbers on the inside indicate interrupted conjugation time intervals in min. Linked genes appear on the same line in clockwise order from left to right.

Transductional analysis of the methionine mutants showed that their sites of mutation fell into 6 closely-linked clusters possibly representing six genes. The topography of *P. mirabilis* mutants is partly in agreement with that of methionine genes in *S. typhimurium* (fig. 2). *MetA* and *H* mutants are linked in both organisms. In *S. typhimurium* the linkage is 35 per cent. (Ayling and Chater, 1968) or 15-22 per cent. (Childs and Smith, 1969) and in *P. mirabilis* 33 per cent. Non-linkage of *metE* and *K* to other *met* mutants is also a feature of *S. typhimurium* (see Sanderson, 1970). *P. mirabilis* differs from *S. typhimurium* in that its *metJ* and *F* mutants are not linked to one another or to arginine mutants. Partial agreement of gene topography in *P. mirabilis*, *S. typhimurium* and *E. coli* has also been reported for arginine genes (Prozesky, 1968, 1969). Genetical differences between *P. mirabilis* and *E. coli* or *S. typhimurium* are expected (Marmur, Falkow and Mandel, 1963) since their guanine+cytosine molar contents are 38-41 per cent., 50 per cent. (Hill, 1966) and 50-52 per cent. (Marmur *et al.*, 1963), respectively.

5. Summary

1. The nutritional requirements of auxotrophs of four possible structural genes indicate that *Proteus mirabilis* may synthesise methionine *via* two pathways. One is that of *Salmonella typhimurium*, *Escherichia coli* and *Pseudomonas aeruginosa* while the other involves S-methylcysteine as in *Neurospora crasa*.

2. Growth requirements of *metE* and *H* auxotrophs indicate that *P. mirabilis* methylates homocysteine by the two routes of *S. typhimurium* and *E. coli*.

3. The properties of two groups of mutants resistant to inhibition by methionine analogues suggests that the regulation of methione synthesis in *P. mirabilis* may be similar to that in *S. typhimurium*. Methionine auxotrophs deficient in D-methione utilisation were not encountered.

4. The genetical topography of the *P. mirabilis* mutants corresponds to that of *S. typhimurium* in that *metA* and *H* are co-transducible and *metE* and *K* are not linked to other *met* mutants. Differences are that *metJ* and *F* are not co-transducible and they are not linked to the arginine cluster in *P. mirabilis*.

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