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Mapping of the *chl*-B Gene in *Salmonella typhimurium* LT2*

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Summary. Preliminary genetic studies of pleiotropic *chl*-r mutants of *Salmonella typhimurium* LT2 were performed, which lead to the description of a new *chl* locus at 123 min on the chromosomal map. By analogy with *Escherichia coli* K12, it is called *chl*-B.

Introduction

In *Salmonella typhimurium* LT 2, nitrate-reductase A (NR A), tetrathionate-reductase (TTR) and formic-dehydrogenase (FDH) can be lost separately by specific mutations affecting only one of these activities (unpublished data; F. Casse *et al.*, 1972; M. Chippaux *et al.*, 1972) or together by single pleiotropic mutations. Several genes in which mutations have such a pleiotropic effect were described in the *gal* region by Stouthamer (1969).

In this paper, we describe pleiotropic *chl*-r mutations mapping in a new locus.

Material and Methods

a) Strains. All the described mutants were obtained from strain number 638 of our collection, and *str*-r derivative of *Salmonella typhimurium* LT 2, SL1092 (P. H. Makela). Spontaneous mutants were selected using the chlorate method described by Piechaud *et al.* (1967). The genotype and sex type of strains used for the mapping are described in Table 1.

b) Mating Experiments. Exponential cultures of both Hfr and F^- strains were mixed in the relative proportion of one donor for 10 recipient cells. This mixture remains for two hours at 37° C without agitation and is then concentrated by centrifugation; small volumes of the concentrated suspension are spread on appropriate selective plates.

c) Transduction Experiments. The exponential culture of the recipient strain was placed for 10 minutes at 37° in contact with the lysate of phage P22 grown on the appropriate donor strain; the ratio phage/bacteria was 1:1.

d) Media. Selection of recombinants was performed on:

— minimal medium containing per liter: carbon source, 2 g; required amino-acids or bases, 40 mg.

— EMB complex medium containing 10 g/l of sugar.

When necessary, streptomycin was added to the final concentration of 100 μg/ml.

The production of nitrite was determined by the Griess Isolway reaction.

The accumulation of H_2 was tested in Durham tubes containing glucose supplemented rich medium.

The ability to reduce tetrathionate was determined on EMB medium supplemented with tetrathionate as described by Le Minor *et al.* (1970).

e) Enzyme Assays. NR A, TTR, formic hydrogenlyase (FHL), FDH and hydrogenase (HYD) activities were estimated according to Pichinoty and Chippaux (1969).

* The study reported here will form part of thesis to be submitted by F. Casse to the Faculté des Sciences of Marseille in 1973 for Doctorat ès Sciences (C.N.R.S. n° A.O. 7027).

Table 1. List of strains

No	Polarity	Genotype	Origin
630	Hfr H10	*rfx*	SH3052 P. H. Makela
631	Hfr H1	*met aro rfb str-r*	SH3585 P. H. Makela
632	Hfr H2	*met aro str-r*	SW1403 P. H. Makela
633	Hfr H7		SH633 P. H. Makela
683	F^-	*his trp gal xyl str-r*	SL1092/str P. H. Makela
667	Hfr K3	*ser*-A *rfa*	SA486 Ph. Hartman
669	Hfr K6	*ser*-A *rfa*	SA536 Ph. Hartman
670	Hfr K2	*pur*-E	SA540 Ph. Hartman
714	Hfr K10	*ser*-A	SA722 K. E. Sanderson
717	F^-	*his trp gal xyl str-r chl*-B	$638/ClO_3$
718	F^-	*his trp gal xyl str-r chl*-B	$638/ClO_3$
783	F^-	*pur*-C *pur*-I *pro*-A *ilv*-A *met*-E *fla rha cya str*-r	SA1361 K. E. Sanderson

Results and Discussion

A total of 97 independant chlorate-resistant mutants were selected under anaerobic conditions. All were pleiotropic, having lost simultaneously NR A, TTR, and FHL activities.

They were mated with Hfr H1 (Fig. 1). At least 200 gal^+ recombinants were reisolated from each mating, and tested for their ability to reduce nitrate, tetrathionate and to accumulate H_2.

For 95 of the 97 chl^- studied mutants, chl^+ unselected recombinants were found among gal^+ selected ones; as linked to *gal*, genes affected belong apparently to the *chl*-A region previously described in *Salmonella typhimurium* by Stouthamer (1969) and corresponding to the similar region described in *E. coli* K 12 by Puig *et al.* (1969). These mutants were discarded; we only studied further the two mutations whose percent of cotransmission with *gal* was < 0.2 when using Hfr H1 as donor.

Enzymatic activities of these two mutants (strains 717 and 718) and of the wild type *chl*-s strain (638) are reported in Table 2. Like in other pleiotropic mutants, the loss of FHL activity is due to the loss of FDH activity, HYD being unaffected.

Strains 717 and 718 were therefore mated with different Hfr strains. No linkage was found between unselected *chl* marker and selected *gal*, *trp* or *his* markers when using Hfr H1 or Hfr H2; these results (Table 3) exclude the 0 to 80 min region as a possible location for these two *chl* mutations.

Having only one marker (*xyl*) between 80 and 138 min we used several Hfr strains whose origin and direction of transfer cover this region.

As shown in Table 3, chl^+ unselected recombinants were found when using Hfr H 10, H 7, K 6 or K3 as donors, and none when using Hfr K2 or K10.

These results indicate that the mutations of strains 717 and 718 are located between the origins of transfer of Hfr H10 and Hfr K10, that is to say between 123 and 130 min on the chromosomal map of *Salmonella typhimurium*.

P22 mediated transduction experiments were performed to locate the mutations more precisely.

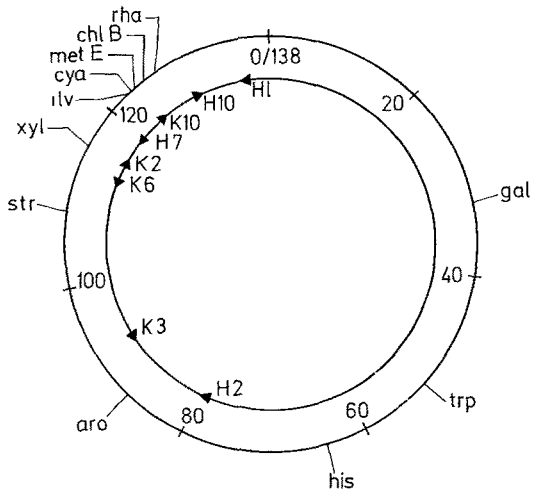

Fig. 1. Chromosomal map of *Salmonella typhimurium* (Sanderson, 1970). The outer circle shows the location of the used markers. Origins and directions of transfer of Hfr strains are indicated on the inner circle

Table 2. Specific activities

Strains	NR A	TTR	FHL	FDH	HYD
638	146	85	9.2	8	186
717	1.9	0	0	0.49	191
718	1.5	0	0	0.25	237

Activities are expressed in μmoles of substrate/h/mg of proteins except for FHL which is expressed in μmoles of substrate/h/mg of cells (dry weight).

Table 3. Results of mating experiments

Donor strain	Sex type	Selected recombinants	Percent of *chl*$^+$ unselected recombinants	
			Recipient 717	Recipient 718
631	H1	*gal*$^+$*met*$^+$*aro*$^+$	<0.2	<0.2
		trp$^+$*met*$^+$*aro*$^+$	<0.2	<0.2
		his$^+$*met*$^+$*aro*$^+$	<0.2	<0.2
632	H2	*his*$^+$*met*$^+$*aro*$^+$	<0.2	<0.2
		trp$^+$*met*$^+$*aro*$^+$	<0.2	<0.2
		gal$^+$*met*$^+$*aro*$^+$	<0.2	<0.2
667	K3	*xyl*$^+$*str*-r	30	2?
		gal$^+$*str*-r	20	22
669	K6	*xyl*$^+$*str*-r	34	50
		gal$^+$*str*-r	25	28
670	K2	*xyl*$^+$*str*-r	<0.2	<0.2
633	H7	*gal*$^+$*str*-r	7	10
714	K10	*xyl*$^+$*str*-r	<0.2	<0.2
630	H10	*xyl*$^+$*str*-r	90	95

Strain 783 was used as a recipient in transductions with P22 lysates of these two *chl*-r mutants. No cotransduction was found between *ilv* and *chl* markers; among the *met* E^+ selected transductants 10% were cya^+ and 4% were chl^- with either 717 or 718 as the donor; moreover none of those which were cya^+ had received the chl^- allele. This indicates that the two studied chl^- mutations are near *met* E but out of the *cya-met* E segment. We can thus postulate that the relative order of the genes is: *ilv-cya-met E-chl*; at the present time these two mutational *chl* sites cannot be genetically distinguished.

We propose *chl*-B as a genetic symbol for these mutations, by analogy with the *E. coli chl*-B gene previously mapped in the homologous region (F. Casse, 1970).

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