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SEARCH OF AUXOTROPHIC MARKERS LINKED TO "Fla$_1$", AND ESTIMATION OF THE FREQUENCY OF THE LINKED TRANSDUCTION BETWEEN "Fla$_1$" AND "H$_1$" IN SALMONELLA.

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In order to study the genetic mechanism of phase variation of flagellar antigen in Salmonella, auxotrophic marker linked to Fla$_1$, which in turn links to H$_1$, was searched by the transduction experiment using Fla$_1^+$-H$_1^i$-auxotrophic (X^-) strains as donor and Fla$_1^-$-H$_1^b$-prototrophic strains as recipient. At the same time, the frequency of the linked transduction of H$_1$ to Fla$_1$ was estimated.

Materials and Methods.

The filtrable agents (FA) of donor strains, which were kindly provided by Dr. Demerec, are listed in Table 1. As recipient strains, penassay broth cultures of S. paratyphi SW-666 (Gal^-, X^+, Fla$_1^-$, H$_1^b$) were used for Gal^+ donor and SW-548 (Gal^+, X^+, Fla$_1^-$, H$_1^b$) were used for Gal^- donor.

FA suspension and recipient cell culture were mixed at the rate of FA:r.c.c. = 1:1 to 5:1 according to the efficiency of FA for Fla$_1^+$ transduction. The mixture was brushed on the motility-gelatine-agar plate or the motility-agar plate, and each swarm, developed after 10 to 15 hours' incubation by the transduction of Fla$_1^+$, was isolated onto EMB-galactose plate or EMB-lactose plate. Its auxotrophism was tested by the replica plate method.

Antigen type was identifyed by slide agglutination test for anti-i serum and anti-b serum.

Experimental Results.

The results obtained are summarized in Table 2.

33 auxotrophic markers tested, involved in 26 strain, are not linked with Fla$_1$, accordingly with H$_1$, except one undecided strain gal-All. FA of the strain

NATIONAL INSTITUTE OF GENETICS
YATA 1111, MISIMA, SIZUOKA-KEN
JAPAN

gal-A11, used in this experiment, has very low transducing efficiency and the enough numbers of swarm for deciding the linkage relation were not obtained.

The efficiency of Fla_1-transduction differes remarkably between some strains (Table 3). The high efficiencies of newly obtained FA of hi-C30, tryp-C3 and cys-A1 compared with the old (a and b in Table 3) suggest that the differences of the efficiencies are attributed to the different degree of degradation of phage activity during storage rather than the specificities of each donor strain.

The frequency of linked transduction of Fla_1 and H_1 is 0.10 of total Fla_1 transduction, and there is no significant difference of the frequency between different donor and recipient strains.

Plan of Farther Experiment.

It is needed to obtain new auxotrophic mutans from Fla_1^+-X^+ strain. For the screening of the mutants, penicillin method will be applied conveniently. The search of marker linked to Fla_1 will be performed using those mutants by the method as same as present experiment and also by the screening of auxotroph linked to Fla_1 immadiately from mass culture.

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NATIONAL INSTITUTE OF GENETICS
YATA 1111. MISIMA. SIZUOKA-KEN
JAPAN

Table 1

FA of auxotrophic mutants of Salmonella typhimurium LT-2 (Fla_1^+ H_1^i), used in the experiment.

No.	FA from strain	Approximate titter	Additional markers
1-a	cys-A1	5×10^{10}	
1-b	"	1×10^{10}	
2	cys-D6	5×10^{10}	
3-a	tryp-C3	1×10^{10}	
3-b	"	"	
4	tryp-D4	"	
5	pro-A6	"	
6	pro-B7	"	
7	ser-A2	"	
8	ser-B10	"	
9	ad-A1		
10	ad-E10		
11	ad-B12		
12	ad-C2		
13	ath-B6		
14	me-A13	5×10^{10}	
15	me-B20	"	tryp-A8
16	me-C1	"	
17	me-D27	1×10^{11}	ad-3
18	me-E2	"	cyst-C7
19	hi-A11	5×10^{10}	cyst-B12
20	hi-D8	"	
21	hi-E22	"	cyst-B12, tryp-A8
22	hi-F31	"	tryp-B2
23	hi-G10	"	
24-a	hi-C30	"	tryp-7, gal-24
24-b	"	1×10^{10}	" "
25	gal-A11	"	
26	gal-B21	"	

cys=cystine, tryp=tryptophan, pro=proline, ser=serine, ad=adenine, ath=adenine-thiamine, me=methionine, hi=histidine, gal=galactose.

NATIONAL INSTITUTE OF GENETICS
YATA 1111. MISIMA. SIZUOKA-KEN
JAPAN

Table 2

Transduction from $Fla_1^+-H_1^i$ auxotrophic strains to $Fla_1^--H_1^b$ prototrophic strains.

No.	Donor strain ($Fla_1^+H_1^iX^-$)	Recipient strain ($Fla_1^-H_1^bX^+$)	No. of swarm tested	No. of auxotroph	No. of antigen b	No. of antigen i	Ratio of i (=frequency of linked t.d. of Fla_1 and H_1)
1-a	cys-A1	SW 666 (Gal^-)	11	0	10	1	0.09
1-b	"	"	39	0	35	4	0.10
2	cys-D6	"	50	0	47	3	0.06
3-a	tryp-C3	"	7	0	7	0	0.00
3-b	"	"	43	0	42	1	0.02
4	tryp-D4	"	50	0	46	4	0.08
5	pro-A6	"	50	0	46	4	0.08
6	pro-B7	"	50	0	40	10	0.20
7	ser-A2	"	50	0	47	3	0.06
8	ser-B10	"	50	0	44	6	0.12
9	ad-A1	"	50	0	43	7	0.14
10	ad-E10	"	50	0	43	7	0.14
11	ad-B12	"	50	0	44	6	0.12
12	ad-C2	"	50	0	43	7	0.14
13	ath-B6	"	50	0	44	6	0.12
14	me-A13	"	50	0	44	6	0.12
15	me-B20	"	73	0	64	9	0.12
16	me-C1	"	50	0	44	6	0.12
17	me-D27	"	120	0	105	15	0.13
18	me-E2	"	50	0	44	6	0.12
19	hi-A11	"	50	0	48	2	0.04
20	hi-D8	"	50	0	47	3	0.06
21	hi-E22	"	50	0	48	2	0.04
22	hi-F31	"	50	0	46	4	0.08
23	hi-G10	"	50	0	46	4	0.08
24-a	hi-C30	SW 548 (Gal^+)	36	0*	30	6	0.17
24-b	"	"	21	0*	19	2	0.10
25	gal-A11	"	2	0*	2	0	0.00
26	gal-B21	"	50	0*	47	3	0.06
Total			1352	0	1215	137	0.10

* include number Of Gal^-.

APPENDIX

Test of homogeneity of the frequency of linked transduction of H_1 to Fla_1.

$X^2 = 24.88$ calculated from the Brandt and Snedecar's formula.

$n = 28$, $0.5 < P < 0.7$

NATIONAL INSTITUTE OF GENETICS
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JAPAN

Table 3

The differences of the efficiency of Fla_1-transduction between different FA.

Strain	Relative yield of swarm*
cys-A1-a	0.046
cys-A1-b	2.3
cys-D6	0.11
tryp-C3-a	0.14
tryp-C3-b	1.3
tryp-D4	1.1
pro-A6	4.4
pro-B7	3.0
ser-A2	0.25
ser-B10	5.4
me-A13	0.48
me-B20	1.5
me-C1	0.44
me-D27	0.55
me-E2	0.74
hi-A11	1.6
hi-D8	0.98
hi-E22	1.3
hi-F31	2.9
hi-G10	0.80
hi-C30-a	0.20
hi-C30-b	1.9
gal-A11	0.044
gal-B21	0.64

* "Relative yield of swarm" corresponds to the number of swarm developed when one loopful (ca. 1.5×10^{-3} ml) of mixture of FA (approx. titter 1×10^{10}) and recipient cell culture was brushed (FA:r.c.c.=2:1).