

FURTHER ANALYSES OF MONOPHASIC NATURE OF STRAIN SW1061
(PSEUDO-MONOPHASICS IN A STRAIN OF SALMONELLA TYPHIMURIUM)

Report 1956-1

by

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In "Report 1956-h", Fla⁻ = 1.2 variations were described on phase 2 monophasic variant SW1061 (-:1.2) of Sal. typhimurium strain TM2 (i:1.2). The transductional analyses have suggested that H₁ in SW1061 is inactive, and Fla⁻ = 1.2 change corresponds to phase variation genotypically; that is the change of the state of H₂.

The inactivation of H₁ may have occurred either by a change of H₁ itself or by an inhibitory change of an adjacent factor against H₁ function. The present report concerns about experimental tests of these alternatives.

MATERIALS AND METHODS.

The experiments consist of two transductions, from Sal. typhimurium SW1061 (-:1.2) to Sal. heidelberg SW1092 (Fla⁻, r:1.2) and from Sal. abony SW803 (b:enx) to SW1061.

General methods of transductions are same as described by Lederberg and Iino (1956).

For the transduction from SW1061 to SW1092, lysates of SW1061 were prepared from 1.2 phase cultures. Two lysates, each of which was prepared from a different single colony culture, were used for the present experiment. As recipients, five overnight penassay broth cultures of SW1092, prepared from single colonies each, were used. Fla⁻-transductions were screened on MGA-plates and their antigens were tested by slide agglutination. When it is necessary, alternative phases were screened by antiserum-MGA deep tubes. Monophasics -:1.2 were confirmed both by non-swarm production on anti-1.2 MGA deep tubes and by segregation of motile and non-motile colonies on MGA plates.

The second transduction SW803 -x SW1061 is the repetition of the same experiment with the transduction in "Report 1956-h", except equal size of the recipient culture was used as a control, to compare the frequency of i:1.2 type production between lysate-treated and non-treated cultures.

EXPERIMENTAL RESULTS.

The results were summarized in Table 1 and Table 2. In transduction from SW1061 to SW1092, most of the types are r:1.2 which were produced by the transduction of Fla₁₀₉₂⁺. Besides r:1.2 type, a few number of -:1.2 and i:1.2 types were recovered. Phase 1 culture of i:1.2 type obtained shows the same intensity of agglutination by anti-i (react to 1/12800 dilution of the original anti-i serum) as phase 1 culture of diphasic Sal. typhimurium TM2. *:1.2 type does not agglutinate even by 1/10 anti-i serum like original SW1061. The ratio of i:1.2 among transductions is high enough to eliminate the possibility of its origin by reversion. It may be explained better by assuming that "the factor which inactivates H₁ function in SW1061 is linked closely but not identical with H₁ itself".

The non-recovery of (i):1.2 type among i:1.2 may be explained by the small size of sampling, as χ^2 test suggests that there is no significant interdependence between the phase of recipient and the frequency of i:1.2 ($\chi^2=5.8$, $0.2 < P < 0.3$). A point remained in question is the low frequency of -:1.2 production compared to the other cases of linked transduction of Fla₁₀₉₂ with H₁. The difference of the frequency ~~of the frequency~~ of the linked transduction Fla₁₀₉₂-H₁ between different cultures has been observed in other cases (Report 1955-b, and unreported data 1956). Low yield of -:1.2 type in the present experiment may also be considered as a case of such diversities of the frequency of the linked transduction.

In transduction from SW803 to SW1061, the number of i:1.2 produced is

larger in the treated than in the control. However, the difference of the frequency is not definitely significant statistically ($\chi^2 \cong 1.8$, $P < 0.2$). The data may be explained as follows: i:1.2 type, at least a part of which, is produced as a result of transduction, but the frequency of reversion from -:1.2 to i:1.2 is about the same as the frequency of i:1.2 production by transduction and it made the difference between the treated and the control insignificant.

DISCUSSION.

- The production of monophasics from diphasic salmonella may occur by
- (1). deficiency or inactivation of H_1 ,
 - (2). deficiency or inactivation of H_2 , or
 - (3). stabilization of H_2 state.

The second and the third cases have already been mentioned (Lederberg and Iino, 1956). The monophasics of SW1061, reported in this paper, belongs to the first case. A remarkable feature of (1) is that flagellated cells appear mixed with non-flagellated cells and interchange each other just as phase variation, contrary with (2) and (3) in which cultures are composed of only flagellated cells of single phase.

Phase variation is defined genotypically as a change of H_2 state. The genotypic change of flagellation in SW1061 is also explained by the change of H_2 state, differed from phase variation only in a point that SW1061 has inactivated H_1 instead of active H_1 . In other words, SW1061 is monophasic serotypically but diphasic genotypically. The term pseudo-monophasics is applied to such type.

The difference of motility between phase 1 and phase 2 of the same strain is not a rare event (Edwards et al. 1954). One of such cases reported

by Seligmann et al. (1945) and Edwards et al. (1954) is quite similar with the case reported here, except phase 1 is not completely non-motile but poorly motile and faintly agglutinable by anti-i. SW1061 may be understood as an extreme type of such cases, although transductional analysis has not been done except SW1061.

The inactivation of H_1 in SW1061 is not caused by the change of H_1 itself but the change of a factor closely linked to it. The function of the factor is not antigen type specific but phase specific. Since it suppresses the production of flagella only in phase 1, the factor is symbolized by Fla_{h1}^- , and its allele in diphasic strain by Fla_{h1} . Fla_{h1} links most closely to H_1 among the factors on which the linkage with H_1 has been observed. It is interesting that the markers linked to H_1 , since discovered, are all "Fla", and among them the most closely linked one is specially related to H_1 function.

SUMMARY.

1). Monophasic strain SW1061 of Sal. typhimurium perform $Fla^- = 1.2$ change in its culture.

2). Transductional analyses indicated that H_1 in SW1061 is inactive, and the change $Fla^- = 1.2$ occurs by the change of H_2 state, correspond to phase variation.

3). The inactivation of H_1 is not caused by the change of H_1 itself but by other factor Fla_{h1}^- which is linked closely to H_1 .

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Table 1.

Transduction from SW1061 (-:1.2) to SW1092 (Fla⁻, r:1.2).

Fla₁₀₉₂ are used as selective marker.

Lysate no. of donor	I	I	II	II	II	Total
Clone no. of recipient	1	2	3	4	5	
Antigen type						
(r): 1.2	0	78	32	2	40	152
r : (1.2)	110	40	6	31	2	189
of						
(i): 1.2	0	0	0	0	0	0
transductions						
i : (1.2)	3	1	0	2	0	6
(-); 1.2	0	5	2	1	1	9
Total	113	124	40	36	43	356
Phase of recipient	1	1&2	2	1	2	1&2

Table 2.

Transduction from SW803(b:enx) to SW1061(-:1.2).

Transductions were selected by anti-b and -enx.

Antigen type	Number of swarms	
	Treated	Control
(-):enx	84	0
b : (1.2)	95	0
i : (1.2)	4	1
Total	183	1