PHASE-1 MONOPHASIC VARIANTS OF Sal. typhimurium AND Sal. paratyphi B

A type of phase-1 monophasic variant occurres by Sh_2^-. Theoretically the other types may occur by the suppressor of H_2, or by a factor which shifts phase-1/phase-2 equilibrium extremely to phase-1 side; in other words, by a factor which inhibits the change from inactive H_2 to active H_2 but does not reverse. The deficiency of H_2 can also be the cause of phase-1 monophasics. These monophasic factors could be in H_2 itself, in H_1, in other locus or in cytoplasm. When phase-2 culture of typical diphasic strain d_1:d_2 is used as a donor and a phase-1 monophasic strain r_1:(r_2) as a recipient of H-transduction, different types of recombinant are expected from the strains with different monophasic factors as shown in table 1. The report concerns with a survey of monophasic factors in phase-1 monophasic variants of Sal. typhimurium and Sal. paratyphi B.

Materials and Methods.

Fifteen i-monophasic variants of Sal. typhimurium and nine b-monophasic variants of Sal. paratyphi B are used for the experiment. They were originally isolated from nature and were identified serotype and monophasic character at C. D. C.

By preliminary test of motility and the frequency of reversion in NGA deep tube, two very weak motile strain and six highly reversible strains were excluded from farther experiment. Phase-2 culture of SW925 a:e,n,x derived from Sal. abony b:e,n,x ---x Sal. sendai a:1,5 was used as a donor. NGA plates supplemented 1/1000 dilution of anti-i serum (for Sal. typhimurium) or anti-b serum (for Sal. paratyphi B) were used for the screening of transductional clones. A phase-1 culture of diphasic Sal. typhimurium TM2 was used also as a recipient to compare the frequency of H_1 and H_2 transduction.

Experimental results.

The results were summarized in table2, which indicates the followings.

(1). H_2 is transduced to all strains tested. Consequently, they have H_2 locus.

(2). In 12 out of 24 strains tested, original phase-2 antigen (1,2) are recovered either by reversion or by transduction. That is, at least 12 phase-1 monophasic

variants have hidden $H_2^{1,2}$.

(3). Except one transduction, --x SW1172, predominant type is $\underline{d_1}:(r_2)$, followed by $r_1:\underline{d_2}$. In 5 among 16 strains, $r_1:\underline{r_2}$ was obtained in small numbers. Therefore, the inhibition of $H_2^{1,2}$ activity occurred either by inactivation of $H_2^{1,2}$ itself or by a factor closely linked to H_2.

(4). The number of transductional clones obtained from --x SW1172 is only 2 (both $r_1:\underline{r_2}$). Whether SW1172 belongs to the same category as (3) or not will be decided after the more number is obtained by repeated experiment. (see also (6) and the later description)

(5). With one exception, number of $r_1:\underline{d_2}$ is considerably smaller than $d_1:(r_2)$. The difference can not be observed when diphasic strain TM2 is used as a recipient. The reason is not clear yet. One possibility is that H_2 and Ah_2 are not linked closely, and H_2 in the recipient is mostly inactive.

(6). The number of $r_1:\underline{r_2}$ type obtained is very small. The experiment will be continued with SW1167, SW1169, SW1172 and SW1178, which may have H_2 inhibitors, Ah_2^-, as monophasic factors. The transduction from SW925 will be repeated on these strains to confirm the constant recovery of $r_1:\underline{r_2}$ type.

SW1167, SW1169 and SW1178 are not sensitive to PLT22. The screening of sensitive mutants is on the way.

From SW1172, a lysate was prepared and was used as a donor of transduction to SW725 $\underline{a}$:e,n,x, SW1167 and SW1178. The results are shown in table 3. SW1172 can transduce both H_1^b and $H_2^{1,2}$ to diphasic^a strain and produces only diphasic type. Consequently, Ah_2 in SW1172 is neither linked to H_1 nor identical with H_2. The linkage to H_2 must be examined by farther transduction experiments with a diphasic strain as a donor. The transductions to SW1167 and to SW1178 produced diphasic^a i:$\underline{1,2}$ types as well as monophasic^a $\underline{b}$ type. This suggests that Ah_2^- in SW1172 is different from monophasic factors in SW1167 or in SW1178.

Table 1.

Transductional types expected from $d_1:\underline{d_2}$ ---x $r_1:(r_2)$.

Location of a monophasic factor	Transductional types							
	$\underline{d_1}:(r_2)$	$(d_1):\underline{r_2}$	$(r_1):\underline{d_2}$	$(r_1):\underline{r_2}$	$\underline{d_1}:r_2$	$d_1:\underline{r_2}$	$r_1:\underline{d_2}$	$r_1:\underline{r_2}$
H_2 deficiency	+	-	-	-	-	-	-	-
on or linked to H_2	+	-	-	-	-	-	-	-
on or linked to H_1	-	-	-	-	-	±A	-	-
other locus than H_1 or H_2	+	-	+S	-	-	-	+K	±A
cytoplasmic	+	-	-	-	-	-	-	-

+: obtained regardless the nature of a monophasic factor.

+S: a H_2-stabilizer causes monophasics.

+K: obtained when a factor which shifts phase equilibrium causes monophasics. It gradually changes to $\underline{r_1}:(d_2)$.

±A: obtained when H_2 inhibitor is H_2 suppressor, and H_2 in the recipient is in active state.

Table 2

Transductional types obtained from SW925 a:e,n,x ---x Phase-1 monophasic variant of Sal. typhimurium or of Sal. paratyphi B.

Serotype	SW-number	Antigen type in phase-1,	phase-2	Reversion to diphase	Transductional clones d_1:(r_2)	r_1:d_2	r_1:r_2	Swarm in control
typhimurium	435	i	(1,2)	frequent	/	/	/	/
"	965	"		none	weak motile			
"	1165	"		none	29	8	0	0
"	1166	"		none	23	4	0	0
"	1167	"	(1,2)	none	22	11	1	0
"	1168	"	(1,2)	frequent	/	/	/	/
"	1169	"	(1,2)	rare	27	3	2	0
"	1170	"		none	weak motile			
"	1178	"	(1,2)	none	21	5	2	0
"	1179	"		none	24	6	0	0
"	1180	"		none	23	1	0	0
"	1181	"		none	20	4	0	0
"	1182	"	(1,2)	frequent	/	/	/	/
"	1183	"	(1,2)	rare	3	6	1	1
"	1184	"		none	23	13	0	0
paratyphi B	705	b		none	59	17	0	0
"	997	"		none	17	10	0	0
"	1164	"		frequent	/	/	/	/
"	1171	"		none	28	6	0	0
"	1172	"	(1,2)	none	0	0	2	0
"	1173	"	(1,2)	frequent	/	/	/	/
"	1174	"	(1,2)	rare	17	5	0	0
"	1175	"	(1,2)	frequent	/	/	/	/
"	1176	"	(1,2)	rare	41	3	0	0
typhimurium	TM2	i	1,2	(diphasic control)	d_1:r_2	21,	r_1:d_2	18.

Table 3

Tranduction from a phase-1 monophasic strain SW1172 b:(1,2)

Donor	Recipient	Screened by	Transductional clone b:e,n,x	Transductional clone a:1,2
SW1172 b:(1,2)	SW725 a:e,n,x	anti-a, & enx NGA	12	24
			b:(1,2)	i:1,2
" "	SW1167 i:(1,2)	anti-i NGA	19	42
" "	SW1178 i:(1,2)	"	11	53