RECURRENT ALTERNATION OF PHASE IN Sal. typhimurium.

Phase variation is a reversible phenomenon. An alternation of phase occurres to both directions, from phase-1 to phase-2 and vice versa. However, the study of phase variation so far has been limited in a few alternative period. Is phase variation perfectly reversible ? Is there no possibility that an alternation affects a manifestation of H-antigen at an unremarkable degree ? Such effect may accumulate in the course of recurrence of the alternation and eventually produce a remarkable change (for example, a change to a monophasic type.). The experiment reported here was designed to look into such questions.

Materials and methods.

Two strains of Sal. typhimurium, TM2 i:1,2 and SW1061 -(i):1,2, were used for the experiment.

Screening of a clone in an alternative phase was performed as follows:

For TM2,

(1). A penassay broth culture (48 hours, 37C) is streaked onto an EMB-gal plate, and it is also subcultured to a penassay medium.

(2). Antigen type of 20 colonies on the EMB-gal plate was examined by slide agglutination test.

(3). A colony with an alternate phase is suspended in saline and is streaked onto an EMB-gal plate. If the colony is not obtained, the subculture in (1) is streaked onto an EMB-gal plate and the procedure is repeated.

(4). A single colony is isolated into a penassay broth.

For SW1061,

(1). A penassay broth culture (37C, 48 hours) is plated with NGA medium.

(2). A clone of an alternative phase, a colony or a swarm, is suspended in saline and is streaked onto an EMB-gal plate.

(3). Antigen type of a colony is checked by slide agglutination test, and the colony is isolated into a penassay media.

(4). When a clone in alternative phase is not obtained in the first NGA plate, a screening procedure is repeated with a subculture.

When the experiment has to be interrupted, the penassay broth culture is kept in refrigerator in both TM2 and SW1061 test.

Experimental results.

The results are summarized in table 1 and table 2. 17 alternations and 35 alternations of phase have been succeeded on TM2 and SW1061 respectively. The tendency to change the frequency of phase variation has not been recognized through the alternations. Their motile phases are as active as the original cultures of both strains.

The experiment will be continued to compare the intensity of agglutination by tube agglutination test and to compare the specificity of antigen type by cross absorption reaction on these clones.

Table 1

RECURRENT ALTERNATION OF PHASE IN TM2 i:1,2.

Alternation number	Antigen type	Number of subcultures	Number of alternative phase per 20 clones	Alternation number	Antigen type	Number of subcultures	Number of alternative phase per 20 clones
1	1,2	1	3	2	i	1	1
3	"	2	2	4	i	3	3
5	"	2	2	6	i	1	2
7	"	2	1	8	i	2	4
9	"	3	3	10	i	1	1
11	"	2	2	12	i	3	5
13	"	2	2	14	i	3	2
15	"	3	3	16	i	3	4
17	"	2	2	18	i		

table 2

RECURRENT ALTERNATION OF PHASE IN SW1061 -(i):1,2.

Alternation number,	from phase	Number of subcultures	Number of clone in alternative phase	total clone tested	Alternation number,	from phase	Number of subcultures	Number of clone in alternative phase	total clone tested
1	1,2	1	54	C	2	-(i)	1	22	141
3	"	1	10	C	4	"	2	48	98
5	"	1	21	C	6	"	1	3	79
7	"	1	36	C	8	"	1	19	65
9	"	1	2	37	10	"	2	100 <	260 <
11	"	1	91	400 <	12	"	1	41	200
13	"	2	24	C	14	"	1	8	26
15	"	2	58	130 <	16	"	1	32	74
17	"	1	6	C	18	"	1	59	148
19	"	1	22	120 <	20	"	1	24	75
21	"	1	19	160 <	22	"	2	13	41
23	"	1	10	54	24	"	1	21	216
25	"	1	2	20	26	"	1	16	39
27	"	2	52	C	28	"	1	1	5
29	"	2	11	76	30	"	1	5	29
31	"	1	25	120 <	32	"	1	9	131
33	"	1	46	C	34	"	1	35	119
35	"	1	4	64	36	"	1		

C: Swarm grew continuously over the surface of a plate.