PHASE VARIATION IN SALMONELLA TYPHIMURIIU M SW629.

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by
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Sal. typhimurium SW629 is the same strain with what has been reported by Seligmann et al. (1945) and by Edwards et al. (1954) as a strain which has poorly motile and faintly anti-i agglutinable phase 1, called X-phase, and normal 1.2-phase 2. The present report deals with the similarity, genetic as well as pheno-type, of the mode of phase variation in this strain with that in SW1061 (Iino, 1956-i).

MATERIALS AND METHODS.

The culture of SW629, used in this experiment, has been maintained in a nutrient agar stab at room temperature.

For the comparative study, Sal. typhimurium strain TM2 (1:1.2), typical diphasics, and SW1061, a monophasic mutant of TM2 with non-motile phase 1 and 1.2-phase 2, were used. In transductional analysis, Sal. heidelberg SW1092 (r:1.2), which has Flav linked to H, was used as a recipient.

Methods of antigen type test and transduction were the same as described in the previous report (Iino, 1956-i). Leifson’s method (Leifson, 1951) was employed for flagellar staining.

EXPERIMENTAL RESULTS.

Antigen reactions of SW629. H-antigen reactions of SW629 were examined followed to the description by Edwards et al. (1954). When the cultures were streaked on FMB-galactose plates, some colonies grown agglutinated with anti-1.2 serum but not with anti-i serum, and the others agglutinated neither anti-i nor anti-1.2 serum (X-colonies of Seligmam et al. 1945).
Microscopical observation of hanging drops and flagellar stains showed that anti-
the cells of anti-agglutinable colony are mostly motile and flagellated, whereas X-phase cells are mostly non-motile and non-flagellated. When two types of colonies were respread on petri dishes with MGA media, anti-1.2 agglutinable colonies formed swarms with few compact colonies, while X-phase colonies formed compact colonies mixed with few swarms. Many compact colonies produced sectorial swarms in prolonged cultures (2 to 3 days at 37°C).

A compact colony (X-phase colony) was isolated from nutrient agar slants. After one or two day culture at 30°C, antigen types were examined by slide agglutination test. As controls, parallel cultures of TM2 originated from i-phase colony and SW1061 from non-motile colony were used. The TM2 culture agglutinated strongly with anti-i serum, but both SW629 and SW1061 didn't. Tube dilution test gave the same result; among dilution series from 1/10 to 1/10x2^12 of original antiserum, i-phase of TM2 agglutinated with 1/10 to 1/10x2^10 antiserums, but SW629 and SW1061 did not show Η-agglutination in any concentrations tested.

The slant cultures were transferred to penassay broth, streaked on EMB-galactose plates, plated on plain or anti-1.2 MGA media, or spotted on anti-1.2 MGA deep tubes. The rest of the cultures were suspended in distilled water and used for flagellar staining. Microscopical observation of the stained bacteria showed that the slant culture of SW629 was the mixture of flagellated and non-flagellated cells. The frequency of non-flagellated cells in a sample was 0.58 (122 among 210). Antigen type of 20 colonies grown on the EMB-galactose plate was as follows; 8 among 20 agglutinated with anti-1.2 serum but not with anti-i serum, and other 12 colonies agglomerated with anti-1.2 serum nor with anti-i serum. On MGA plates, 175 compact colonies and 123 swarms were recovered (the frequency of compact colonies was 0.59, which coincide well with the frequency of non-flagellated cells in the culture), whereas in anti-1.2 MGA plates all of 312 colonies grown were compact; that is,
The swarms in plain MGA plate were 1.2 type. The anti-1.2 deep tube culture of SW629, however, produced swarm which agglutinated distinctly with anti-i serum. The culture from the swarm has maintained 1:1.2 diphasic character in serial transfer through polysaccharide broth.

The observations on slant culture were repeated on three other single colony isolations of the X-phase. They all gave the same result with the culture described above, except that the proportion of phase 2 cells in cultures differed in different slants.

Selection of i-phase by anti-1.2 MGA deep tubes were repeated with larger number of X-phase cultures. Each of 25 single colonies of X-phase and the same number of single colonies of non-motile phase of SW1061 was cultured on an anti-1.2 MGA deep tube. 23 among 25 tubes of SW629 produced swarms which agglutinate with anti-i serum, on the contrary only 6 among 25 produced swarms in SW1061 cultures.

From these results, it is summarized that SW629 is the mixture of Fla- cells and Fla+(1,2) cells, which interchange each other. The interchange is parallel with phase variation in diphasic strain and Fla+(1,2) is Fla-interchange in SW1061. The culture also contains few Fla+(i) cells which are produced by reversion of Fla- cells. The frequency of Fla+(i) production in SW629 is higher than in SW1061, but not high enough as can be recovered without mass selection by anti-1,2 MGA media.

Transductional analyses. A factor which inactivates flagellar production in phase 1 was analyzed by the same method used in transductional analysis of the monophasics of SW1091; that is, SW629 was used as donor and SW1092 as recipient, and Fla1092 transductions were screened from brushes on MGA plates. The results were summarized in Table 1. 3 i:1,2 types and 32 (F):1.2 types were obtained among 332 Fla1092 transductions, besides r:1,2
and (1)1:2 types ( (z):1:2 indicates a type which has Fla− phase 1 and 1:2 phase 2 which reverts to produce 1 phase 1 ). This result is explained by the assumption that the fail of flagellar production in phase 1 is caused not by the genetic change of Hl itself but by the suppression of Hl function by a factor which links to both Hl and Fla1092. The factor will be designated as Flah1-2 tentatively ( and the suppressor in SW1061 as Flah1-1).

In order to test whether Flah1-1 and Flah1-2 are allelic or not, transductions were made between SW1061 and SW629 to both directions. The lysates obtained from SW629(-), SW1061(-) and TM2(i) were diluted to the same titer, 2x10^9/ml. 0.25 ml of each lysate was mixed with 0.25 ml of penassay broth culture of SW626(-) or SW1061(-) (density of the cells was 10^9/ml), and brushed on anti-l.2 MGA plates. The number of swarms recovered was listed in Table 2. Antigen type of the swarms, sampled 20 clones from each combinations, were tested after isolating to EMB-plates. They were all i.

In combinations in which SW629 is the recipient, 68 swarms were recovered when SW629 is the donor. These swarms may be appeared not by transduction but by reversion. The number of swarms increased when SW1061 is used as donor, and became highest when TM2(i) is donor. When SW1061 is the recipient, none of the swarms were recovered from self-transduction, whereas 23 swarms appeared in SW629-x, and more than hundred in TM2-x. These results indicate that Flah1-1 and Flah1-2 are not allelic but linked closely. The production of trails were observed only when TM2 is donor. The significance of this phenomenon will be discussed later.

Stability of Flah1-1 and Flah1-2. As the result of i-phase screening by anti-l.2 MGA stabs has indicated, SW629(-) revert more frequently to 1:1.2 type than SW1061. The difference of the frequency was also observed between -1:1.2 clones obtained from SW629-x SW1092 and SW1061-x SW1092.
In Sw628 -x Sw1092, 30 among 32 1:1.2 transductional clones produced phase 1 swarms in the first selection, and the other two produced in the second selection; whereas 1:1.2 from Sw1061 produced only two reversions among five repeated selections of five 1:1.2 clones. Consequently, the difference in the frequency of reversion between Sw629 and Sw1061 is attributed to the different reversibility of Flah1-1 and Flah1-2.

DISCUSSION.

Phase variation of Sal. typhimurium Sw629 is performed between Fla- phase 1 and 1.2-phase 2. The strain also produces 1:1.2 diphasic type by reversion. These behaviors coincide well with the description by Seligmann et al. (1945) and Edwards et al. (1954) except one point that the definite i reaction could not be observed on nutrient agar slant cultures in this experiment. The discrepancy on the reaction of agar slant culture may be explained by either one of following two reasons. The first is the difference of media used; the composition of the nutrient agar slant used in this experiment may differ from what has been used by Edwards et al., and could not support flagellar production in phase 1 of Sw629. The second explanation is based on the high frequency of reversion of Sw629; Sw629 reverts frequently to 1:1.2 diphasic type, and mass cultures usually contain some proportion of i type cells. The culture reported by Edwards et al. may be the culture which has contained high proportion of i type cells.

Transductional analysis showed, Sw629 has potentially active H1 but its function is suppressed by a factor, Flah1-2. The mode of suppression by Flah1-2 is quite similar with that of Flah1-1 in Sw1061. In both cases, suppression is on H1 function but not on H2. In both cases, the factor linked to H1 and Fla1092. The factors, however, are not allelic and also differ in
their frequency of reversion.

The transductions SW629 × SW1061 on anti MGA media are the first examples in which swarms were obtained but trails were not from transductions between Fla− strains. The explanation of the mechanism of trail phenomena in Fla-transductions still allows several alternatives (Lederberg, 1956). However, it is the least common assumption that the first step of the trail production is the expression of the phenotype (flagellar production) before perfect organization of fla factor into the chromosome of the recipient takes place. Based on this assumption, together with the fact that FlaH1-1 and FlaH1-2 link closely each other, the failure of the trail production is most plausibly explained that Flah1-1 or Flah1-2 can not express their function unless they are arranged in a chromosome together; that is, they have cis-trans position effect each other.

SUMMARY.

1). *Sal. typhimurium* SW629 changes its phase between Fla− (phase 1) and 1.2 (phase 2) in the media tested.

2). The factor (Flah1-2) which suppresses the production of flagella in phase 1 links to H1 and Flah1092, but not identical with the H1 suppressor (Flah1-1) in SW1061. Flah1-1 and Flah1-2 also link closely each other, and may belong to a cistron, but have different frequency of reversibility.

REFERENCES.


Iino, T. Report 1956-1, Further analyses of monophasic nature of strain SW1061.

Lederberg, J., 1956, in press.


Table 1.
Transduction, Sw629 (-:1.2) –x SW1092 (Fla-, R:1.2)
Fla1092 were used as selective marker.

<table>
<thead>
<tr>
<th>Experimental no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Total</th>
</tr>
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<tr>
<td>(r):1.2</td>
<td>35</td>
<td>2</td>
<td>33</td>
<td>50</td>
<td>12</td>
<td>13</td>
<td>145</td>
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<tr>
<td>Antigen type</td>
<td>(r):1.2</td>
<td>29</td>
<td>40</td>
<td>13</td>
<td>3</td>
<td>49</td>
<td>27</td>
</tr>
<tr>
<td>(i):1.2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>(r):1.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>(T):1.2</td>
<td>11</td>
<td>1</td>
<td>7</td>
<td>9</td>
<td>0</td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>43</td>
<td>55</td>
<td>62</td>
<td>62</td>
<td>44</td>
<td>392</td>
</tr>
</tbody>
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Table 2.
Number of swarms and trails produced from transductions among TM2, Sw629 and Sw1061. Swarms were screened on anti-1.2 MCA plates.

<table>
<thead>
<tr>
<th>Donor</th>
<th>TM2</th>
<th>Sw629</th>
<th>Sw1061</th>
<th>TM2</th>
<th>Sw629</th>
<th>Sw1061</th>
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<tr>
<td>Recipient</td>
<td>Sw629</td>
<td>Sw629</td>
<td>Sw629</td>
<td>Sw1061</td>
<td>Sw1061</td>
<td>Sw1061</td>
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<tr>
<td>no. of swarm</td>
<td>218</td>
<td>68</td>
<td>162</td>
<td>114</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>no. of trail over</td>
<td>300</td>
<td>0</td>
<td>0</td>
<td>over</td>
<td>500</td>
<td>0</td>
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