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THE GENETICS OF SALMONELLA

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Our immediate objective is to determine the role that genetic recombination may play in the formation of new types in the Salmonella group. If, as in *Escherichia coli*, recombination should be found in Salmonella, it can then be used as a tool for the analysis of specific genetic problems in this group, in particular, the relationships of somatic antigens, phase variation in flagellar antigens, and determination of virulence.

It is hoped to apply to Salmonella the procedures which worked with *E. coli* as detailed in <sup>1</sup>. Complementary biochemical mutants would be mixed and plated into minimal agar. The development of prototroph colonies with no nutritional deficiencies would be a sign that recombination of factors had occurred.

The progress which may be reported for the period July 1, 1948, to March 1, 1949, consists primarily of (A) the development of an improved technique for the isolation of biochemical mutants, and (B) preliminary recombination experiments on three strains of *S. typhimurium*.

In the exploration of a new bacterial group for gene recombination, most of the labor would be spent on the isolation of the multiple biochemical mutants needed to look for the formation of prototroph recombinants. For this reason, considerable attention was given to the possibility of improving methods for selecting biochemical mutants. Penicillin might be a useful reagent for this purpose since it has been established that this antibiotic has a bactericidal action specific for growing cells. After a number of preliminary reconstruction experiments, a procedure was worked out with which the mutant cells present in small numbers in ultra-violet treated suspensions could be concentrated so as to facilitate their isolation.

The suspensions are inoculated into synthetic medium containing penicillin. The mutants are protected from bacteriolysis by their inability to grow in this medium, while the non-mutant cells are rapidly killed. Details have been published<sup>2</sup>. Just as we submitted this manuscript for publication, we learned that Bernard Davis, Senior Surgeon, U.S.P.H.S., at the Tuberculosis Research Laboratory in New York, had independently developed the same technique. We arranged to publish our findings as adjoining notes in the same journal, and found it economical to reprint the notes on the same sheets.

This method has been applied with considerable success to three *Salmonella typhimurium* strains, with our designations: SY-20, SY-21, and SY-23. SY-20 and SY-21 are monophasic variants, in the first and second flagellar phase respectively, originally received from Dr. P. R. Edwards, while SY-23 is a typical diphasic culture. The strains also differ in their fermentative reactions, and in their phage resistance patterns.

1. Lederberg, J. Gene recombination and linked segregations in *Escherichia coli*. Genetics 32:505-525 (1947).
2. Lederberg, J., and Zinder, N. Concentration of biochemical mutants of bacteria with penicillin. J. Am. Chem. Soc., 70, 4267 (1948).

A number of mutants, and then, double mutants, were produced in strains S-20 and S-21. Using the principle described above, no indication of recombination was found between mutants either of the same or of the two different strains.

Somewhat more encouraging results were obtained with SY-23. However, we have been disappointed to discover that it has not been possible to secure unequivocal evidence for or against the occurrence of recombination in *Salmonella* with the same ease as in *E. coli*.

Combinations of mutants within strain SY-23 have erratically led to the production of prototrophs, particularly in a "cross" of a proline-histidineless with a leucine-methionineless, rough, mutant. In this case, it is unlikely that prototrophs could have originated by reversion of either of the parents, although a syntrophic effect is not excluded.

More consistent results have been obtained in an interstrain cross of an isoleucine-valineless mutant (SW-13, from SY-21) with a histidine-threonineless (SW-25, from SY-23). In about every other experiment, a few prototrophs have been found from this combination. These cultures also differ in the fermentation of maltose, xylose, galactose, and arabinose. The prototrophs which could be tested usually showed the fermentation pattern of the SW-13 parent, although a very few have been like SW-25. However, it was noted during these fermentation tests on indicator agar that the prototrophs isolated from these experiments are frankly lysogenic, i.e., when spread out on nutrient agar, they are ridden with plaques of bacteriophage. In spite of repeated serial single colony isolation, it has not been possible to free the bacterial cultures from this phage. This contamination probably also accounts for the relatively poor growth of these prototrophs in minimal medium, as compared with either of the original wild type parents.

Further study showed that each of the two strains carries, in lysogenic condition, a phage active against the other. SY-21 also carries a second phage which is active on *S. gallinarum*, but not on other typhimurium isolates. It has not been possible to obtain these phages in high titer as yet. Our attempts, so far, to obtain resistant mutants which would be unaffected by either of the phages have also been unsuccessful.

The suicidal behavior of the prototrophs obtained from this experiment makes it very difficult to study them, and therefore to draw any definite conclusions as to the validity of the prototrophs which have appeared in these experiments. Especially perplexing is the fact that prototrophs isolated from the intrastain cross in SY-23 are also suicidal, although both parents were shown to carry, and to be resistant to lysis by, the same phage.

By coincidence, lysogenicity has also become a vital issue in other genetic work being done in this laboratory on the genetics of *E. coli*, with the finding that *E. coli* K-12 is an "immune carrier" of a phage that remained undiscovered until certain cultures were disinfected (and rendered susceptible) by ultra-violet light.

Although lysogenicity probably has no direct relationship to genetic recombination, we are imposed with the obligation to it, (a) as a factor which interferes with "interbreeding", i.e., as an important isolation mechanism, and (b) as a type of interreaction between cultures comparable to type transformation.

For the present, our interest in lysogenicity in Salmonella is confined to its intrusion in our recombination experiments. Attempts to disinfect Salmonella cultures with ultra-violet, along the lines which were successful with E. coli, have not been fruitful so far. However, it has been noted that the action of the lysogenic phages is much less marked at room temperature, and concordantly that the yield of prototrophs is higher at lower temperatures.

The last line of approach is to proceed with the development of other strains, in hopes of escaping interference from phages. However, lysogenicity in this group appears to be exceedingly common ~~in this group~~, and it is doubtful whether one could safely assert that a given culture was not contaminated with a symbiotic phage until it had been exhaustively tested with a very large number of suitable indicator strains.

Incidentally to the genetic studies, a mutant of SY-23 was isolated which has interesting properties from a biochemical point of view. The mutant, SW-38, responds either to phenylalanine or to tyrosine, and preferably to the combination. This pattern suggested that the mutant was deficient in the synthesis of the aromatic ring, so a number of related phenols were tested. Many of these proved to be active, especially in combination with serine, including phenol, hydroquinone, catechol and cyclohexanol. On the other hand, resorcinol and naphthol were inactive. Unfortunately, the mutant is highly unstable, and reversions are selected for, and lead to growth in unsupplemented medium within a relatively short time. However, the response to cyclohexanol plus serine has been verified by plating experiments in which small numbers of cells were added to synthetic agar with various supplements. This finding suggests that the aromatic ring may be synthesized from a saturated cyclic intermediate such as cyclohexanol, and that it is subsequently condensed with an "alanine" residue to form the aromatic amino acids. However, this study is not being pursued further, as it is too far from our primary genetic objectives.