

PROGRESS REPORT

L. Baron

Feb. 21, 1962

Phages of Bacillus Subtilis

Three phage stocks and their host strains were received from P.B. Cowles, isolated from soil. These phages did not produce plaques on the Marburg strain of Bacillus subtilis and derivatives thereof (SB19, SB23, 168).

A series of phages were obtained from Romig, J. Bacteriol. 82:135, 1961. The information concerning the behavior of these phages given in Romig's paper is accurate. The following notes indicate some difficulties encountered.

1. Stocks are best prepared by the "confluent plate lysis" method.
2. Except for phage SP-3, plaques of Romig's series of phages are minute and in some instances, quite difficult to see.
3. Titers of lysates (originally  $10^9$  -  $10^{10}$ /ml.) decrease considerably on storage at 4° C. (to below  $10^3$ /ml. after 1 month storage).
4. Chloroform inactivates all of the phage stocks (to some extent), so that sterilization by filtration is required.
5. It was impossible to obtain stable resistant colonies of SB-19 or 168 to any of the phages, an experience also shared by Romig. Stocks of Romig's phages, SP-5,6,7,8,9,13, and Cowles' phages 04,5,6 are stored in the refrigerator, but are now of low titer. Stable high-titered stocks of Romig's phage SP-3, probably the most useful phage from the standpoint of the above factors, are also in the refrigerator.

Phage SP-3 is described in a paper by Romig to be published in Virology----Contaminated T4 Phage--a preparation of T4 phage originally  $10^{13}$  titer after purification over a year ago was obtained from J. Josse. The preparation was overgrown with over  $10^{10}$  viable cells of a pigment producing strain of Pseudomonas. The viable phage titer was approximately  $10^9$ /ml. DNAase had been added to the original phage suspension.

Transformation Experiments

Transformation of various markers in B. subtilis using a number of heterologous DNA preparations (E. coli, Serr. marcescens, S. typhosa, V. cholerae) was unsuccessful. Ten different markers were tested using competent cells which were transformable with SB-19 DNA. In all instances, inhibition of transformation by SB-19 DNA occurred in the presence of

Baron

heterologous DNA. This might be a more profitable approach using DNA preparations from other transformable species (Hemophilus, Pneumococcus) provided that the melting points of the markers matched.

Transformation of Streptomycin Dependence - a streptomycin mutant of 168 was isolated and DNA prepared from this strain. Procedure for the transformation of the  $S^d$  marker is the same as for transformation of streptomycin resistance. A small number (less than 1 per cent) of the colonies were  $S^r$  rather than  $S^d$  (probably  $S^r$  mutants), although none appeared on the control plates.

Streptomycin dependence to independence did not seem to be a very useful marker for a few reasons. Growth of the  $S^d$  culture was extremely poor in CH-T-1 and CH-T-2 regardless of the amount of streptomycin added; in addition, there appeared to be a fair amount of reversions when the cells were washed free of the streptomycin before plating.

Transformation of episomes to E. coli or Serr. marcescens using interrupted mating mixtures were unsuccessful. Transformation or mating of B. subtilis for Lac or Gal episomes of E. coli were likewise unsuccessful.

Lysozyme Resistance: Mutants resistant to lysozyme up to levels of 5,000/ml. were detected in numbers decreasing asymptotically. These mutants were completely unstable after transfer from the original lysozyme plates to broth. Streptomycin produced about a 30 fold decrease in lysozyme activity probably due to binding of the lysozyme.