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PRELIMINARY REPORT

REDUCTION IN IMMUNITY TO ITS OWN COLICIN ASSOCIATED WITH ANTIGEN SEGREGATION FROM PHASE I TO PHASE II IN A WILD STRAIN OF *SHIGELLA SONNEI*

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Heterogeneity in sensitivity to colicin among daughter colonies has been reported in certain strains of *Shigella*. (ABBOTT *et al.*, 1958, SAMARAKI-LYBEROPOULOU *et al.*, 1965) However, heterogeneity in colicinogeny has not yet been reported. (FREDERICQ, 1957) Since a phase I colony of *Sh. sonnei* continues to segregate phase II progeny colonies, it seems interesting to see whether antigen segregation is accompanied by any changes in colicinogeny. Three wild strains of *Sh. sonnei*, recently isolated in our laboratory from different sources of infection, were studied along this line. It was found that in two of the strains studied phase I cultures produced colicin which was active on their own phase II cultures. This is a preliminary report on only one of these strains which was studied in fair detail.

The strain studied, *Shigella sonnei* 24—66, produced colicin which was active on *E. coli* K 12 Row and *E. coli* B. This strain, however, carried no temperate phage which was active on *E. coli* K 12 Row or *E. coli* B. It was sensitive to antibiotics such as chloramphenicol, streptomycin and tetracyclin. It was spread on a nutrient agar plate and the colonies developed were tested by the agglutination test with absorbed phase-specific antisera. Then the colonies chosen as phase I and phase II were each tested for colicinogenic activity against themselves and against each other. The test was carried out according to the procedures of Fredericq (FREDERICQ, 1958). A small inoculum from one of the colonies was stabbed into a nutrient agar plate, incubated for 24 hr 37°C and sterilized by chloroform vapor. A semi-solid culture of the other colony in agar was then overlaid on the sterilized plate. As shown in the figure, the growth of the phase II culture was inhibited by the colicin produced by the phase I culture and a clear inhibition zone of about 11 mm in diameter was observed in the confluent growth of the phase II culture. The appearance of an inhibition zone was prevented by adding crystalline trypsin to the semi-solid agar at a concentration of 100 μg/ml. The phase II culture showed no inhibition zones against the phase I culture. The phase I culture was not active on itself. A heterogeneity in colicinogeny was thus found among colony phases of a single strain.

To confirm the above observations, isolation of single colonies was repeated. Serial re-spreading of single colonies of the phase I bacteria on nutrient agar continued to result in segregation of phase II daughter colonies with an average frequency of 7 per cent. A number of pairs of phase I and phase II

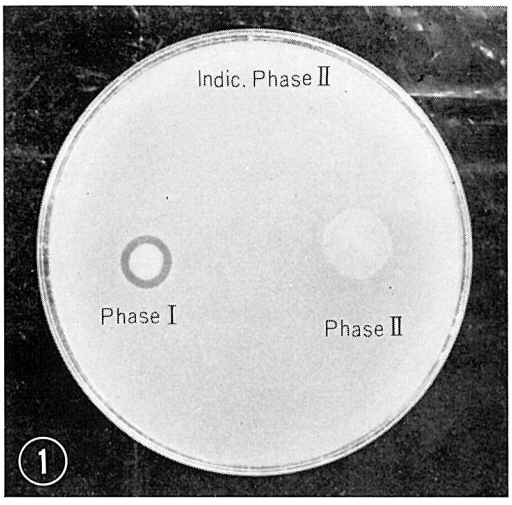

FIGURE 1 A comparison of the inhibition of growth of phase II of *Sh. sonnei* 24–66 by phase I (left) and phase II (right) colonies of the same strain.

colonies on each plate were tested reciprocally for their colicinogenic activity against each other. Consistently reproducible results were obtained in which all the phase II colonies tested were sensitive to the colicin produced by the phase I colonies and no colonies of phase I were sensitive to the colicin produced by the phase II colonies. None of the phase I colonies were found to be active against themselves in parallel tests.

Some colonies on the isolation plates were examined for their responses to some of the standard colicin type test strains. The results were uniform on each plate. Both the phase I and II cultures produced colicins which are active on *E. coli* K 12 Row and both cultures were sensitive to phage BF 23, which is known to be adsorbed on a common receptor of group E colicins. The phase I culture resisted the action of the colicins of *E. coli* K 12–30 (E_1) and *E. coli* K 30 (E_1+ V). Thus the phase I culture was insensitive to colicin E_1, although it had the specific receptor for it. The phase II culture was also insensitive to the colicin E_1 of *E. coli* K 12–30 but it was sensitive to the colicin of *E. coli* K 30. Moreover, the phase I culture resisted the action of colicin produced by *S. typhimurium* TM 2 (B), while the phase II culture did not. Thus the phase I culture differs from the phase II culture in sensitivities to various types of colicin other than E_1.

Next attempts were made to see whether there was any difference in colicinogeny between phase I and phase II cultures. For this, two resistant mutants of *E. coli* K 12 Row were used. One was *E. coli* K 12 Row/E and the other was *E. coli* K 12 Row resistant to the colicin of phase II cultures, which was isolated from the inhibition zones of *E. coli* K 12 Row produced by phase II cultures. Colicin produced by phase I cultures was active on *E. coli* K 12 Row/E while colicin produced by phase II cultures was not. However, colicin produced by phase I cultures was not active on *E. coli* K 12 Row resistant to the colicin of phase II cultures. The latter fact would preclude the possibility that the difference in sensitivity between phase I and II cultures mentioned above was due to any differences in their colicinogeny. It therefore seems reasonable to conclude that phase segregation is related to changes in the cell surface of the bacteria which renders them sensitive to the action of colicins, and even to their own colicin.

As already mentioned, a fresh subculture from a phase I colony did not show any inhibition zones against itself. With phase II colonies the situation was different. The colicinogeny against itself was peculiar and complicated. When a phase II colony was spread on nutrient agar and then a number of the resulting colonies were tested in pairs for their colicinogenic activity against each other, some pairs showed no inhibition zones while others showed clear inhibition zones. Moreover, the clear zones which developed were not necessarily uniform in shape and size. Results were similar on repeated isolation of single colonies of phase II.

There are two possible explanations for this peculiar phenomenon. Formation of

inhibition zones by phase II cultures might be due either to the presence of exceptional variants which are active on phase II bacteria or to the presence of exceptional variants which are sensitive to the colicin produced by phase II bacteria. Survivors in the clear zones might be either active variants or sensitive variants which have been rendered resistant to the colicin produced by phase II bacteria. In the first trials to isolate survivors, the surface of the clear zones was swabbed and streaked on both nutrient agar and SS agar plates. No colonies other than those belonging to phase II were recovered in either case, so that the possibility of a reverse change of phase II bacteria to phase I is unlikely. Next, two well developed, discrete colonies on the clear zones were isolated and submitted to more or less detailed characterization for their colicinogeny. These survivors, which still retained the serological characteristics of phase II, were found to be no longer sensitive to the colicins produced by either phase I or phase II cultures.

These characteristics remained stable even after successive subculture. Their attitudes to the colicinogenic strains of *S. typhimurium* TM 2, *E. coli* K 12 and *E. coli* K 30 as indicators and also to *E. coli* K 12 Row and *E. coli* K 12 Row/E as producers were similar to those of the original phase II culture. Unlike the original phase II culture, they showed no sensitivity to phage BF 23, indicating loss of the E receptor. The simplest interpretation of these results is that the clear zones in the culture of phase II alone are due to exceptional variants which are sensitive to the colicin of phase II cultures. However, the mechanism involved is not yet clear and much work is still required on this problem.

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