ABORTIVE INFECTION OF PSEUDOMONAS AERUGINOSA AND SERRATIA MARCESCENS WITH COLIPHAGE P1

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In the course of attempts to effect intergeneric transduction by means of coliphage P1 (Bertani, J. Bacteriol. 62:293, 1951; Lennox, Virology 1:190, 1955), we observed that this phage can attack and kill many strains of Pseudomonas aeruginosa and Serratia marcescens. Bacterial killing is not accompanied by phage multiplication.

Lysates of phage Pl{k} or its mutant Pl{k} vir, grown on either Shigella dysenteriae Sh or Escherichia coli K-12 derivatives, were tested by spotting loopfuls of serial dilutions on plates of complete or minimal medium seeded with a soft-agar layer containing enough test bacteria to give a continuous layer of growth. The plates were incubated at various temperatures from 20 to 42 C. Inhibition reactions, in the form of clear or partially clear areas, were obtained with 22 out of 47 strains of P. aeruginosa tested. Similar but less reproducible results were obtained by spot tests on plates inoculated by spreading bacteria on the agar surface.

P. aeruginosa strain 1 [try] (Holloway), which gave a typical inhibition reaction, was studied in greater detail. Growth-inhibition areas were observed with amounts of Pl{k} of 10^4 or more plaque-forming units per drop, measured on S. dysenteriae. For a virulent mutant of Pl{k}, only one-tenth as much phage was needed to give a clear inhibition reaction. The inhibition areas were characteristicall different for Pl{k} and Pl{k} vir. Inhibition areas were observed when phage was plated with mixed cultures of S. dysenteriae and P. aeruginosa; the plaques formed on Shigella contained enough phage to inhibit the growth of Pseudomonas. All attempts to transfer inhibitory phage serially from the inhibition areas on P. aeruginosa to the same host or to other host bacteria were unsuccessful.

In liquid medium, phage was adsorbed rather slowly and not exponentially by P. aeruginosa cells, the first 80 to 90% being removed in 30 min by a suspension of 5 x 10^8 bacteria per ml; further adsorption was slower. Bacteria from growing and from stationary cultures behaved similarly. Killing of up to about 70% of the cells was demonstrated by viable counts of mixtures of bacteria with excess phage; no lysis was observed.

The following tests served to inerminate the phage particles as responsible for the killing effect: (i) Killing occurs only in the presence of Ca^{++} ions, which are required for adsorption of phage P1 to bacteria (Bertani, J. Bacteriol. 62:293, 1951). (ii) In electron micrographs of phage-cell mixtures, phage particles were seen attached to cells of P. aeruginosa strain 1 [try] but not to control cells of resistant bacterial strains. (iii) Anti-P1 serum destroyed the killing activity of lysates. (iv) Upon centrifugation, both with full sedimentation and with banding in a CsCl density gradient (Weigle et al., J. Molecular Biol. 1:379, 1959), killing activity went with phage activity and was removed from the fractions not containing phage. (v) Uninfected cultures of E. coli and S. dysenteriae, as well as their supernatants and extracts, had no killing effect on P. aeruginosa; nor did lysates of phages T6 and \( \lambda \), produced on the same cultures as the P1 lysates, give any growth-inhibitory reaction.

No Pl-lysogenic bacteria could be isolated from either the areas of inhibition on agar or the phage-cell mixtures in liquid. To test whether production on P. aeruginosa was masked either because of pH sensitivity or by failure to produce a lysozyme-like lytic enzyme (Campbell, Virology 14:22, 1961), media were prepared at various pH levels from 3.5 to 9.0, with or without added egg lysozyme; no phage production was obtained.

We conclude that phage Pl{k} is specifically adsorbed by some strains of P. aeruginosa and kills some of the adsorbing cells by a process not accompanied by production of recognizable mature phage (abortive infection). The difference in the type of inhibition reaction between Pl{k} and Pl{k} vir suggests that the phage genome enters the bacteria and that at least some of the early reactions of phage development take place. It should be pointed out that the deoxyribonucleic acid (DNA) of phage P1 has the
base composition of typical *E. coli*, while *P. aeruginosa* DNA is quite different (Sueoka, J. Molecular Biol. 3:31, 1961).

A similar inhibition phenomenon, traceable to phage adsorption and bacterial killing without phage multiplication, was observed with five strains of *S. marcescens*. Here the difference between Plk and Plk vir was reversed, the latter being less effective at equal concentrations. Note that *S. marcescens* has a DNA base composition different from either *P. aeruginosa* or *E. coli*, but can support the continued growth of an episome, the fertility factor F, apparently consisting of coli-type DNA (Marmur et al., Proc. Natl. Acad. Sci. U.S. 47:972, 1961).

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AN IMPROVED METHOD FOR THE DETECTION OF SPORE DISCHARGE IN THE SPOROBOLOMYCETACEAE

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In heavily sporulating strains of yeasts belonging to the *Sporobolomyces*, ballistospore formation can be observed easily on the surface of malt agar. Spore discharge is studied commonly by inoculating the yeast on malt agar or on potato glucose agar in a petri dish and inverting the dish. Due to the discharge of ballistospores, a mirror image is formed on the lid below the agar. A pair of slides, separated by a glass support, may be used in the same fashion (Lodder and Kreger-Van Rij, *The yeasts*, North Holland Publishing Co., Amsterdam, 1952). In poorly sporulating cultures, a mirror image is not evident, but the test becomes more sensitive if two bottoms of petri dishes are used, both containing growth medium. The few discharged spores then germinate and form colonies in the bottom dish. A sterile glass slide may be included in the lower dish to collect spores for microscopic observation.

Recently we isolated an interesting new species of yeast (Phaff and do Carmo-Sousa, *in press*). Neither ascospores nor ballistospores could be found. The organism was cream colored and somewhat slimy in appearance, but a starchlike compound (characteristic of species in the genus *Cryptococcus*) was not formed.

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The organism appeared to belong to the genus *Torulopsis* or to *Candida*. Since the formation of pseudomycelium on potato glucose agar slides (Lodder and Kreger-Van Rij, *The yeasts*, North Holland Publishing Co., Amsterdam, 1952) was not decisive and characteristic, the experiment was repeated with corn meal agar, prepared as described by Skinner (Bacteriol. Rev. 11:227, 1947). To our surprise the growth on corn meal agar slides showed the formation of asymmetric ballistospores (genus *Sporobolomyces*). When the yeast was inoculated in petri dishes containing malt agar (5%), potato glucose agar, or corn meal agar, and the dishes were inverted over malt agar, only the culture grown on corn meal agar discharged ballistospores (which formed colonies on the malt agar below). When the colonies growing on malt agar were inverted over another plate of malt agar, no spore discharge occurred.

Subsequently, another culture was isolated, and was initially classified as a species of *Torulopsis*; it produced small numbers of symmetrically shaped ballistospores when grown on corn meal agar. In addition, we had in our collection several strains of *Bullera alba* which appeared to have lost the ability to produce ballistospores when tested by the standard method. On corn meal agar, however, ballistospore discharge could be detected without difficulty. This