Rapid Plate Method for the Isolation of Lysogenic Bacteriophages

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We have devised a rapid plate method for the detection and isolation of lysogenic phages and have used the procedure to screen 15 strains of *Staphylococcus aureus*. This method should prove applicable to a wide variety of bacterial species and should be especially valuable for those pathogens for which no phage-typing system is available.

Screening procedures designed for the isolation of virulent or non-episomal temperate phages usually present no difficulties for the majority of bacterial species. On the other hand, the screening procedures used for the isolation of stably integrated lysogenic phages most often involve induction of broth cultures of individual bacterial strains followed by centrifugation or filtration, or both, to remove cells, and finally spotting of the lysates on possible indicator strains. The need for these manipulations severely limits the number of samples that can be conveniently handled within a reasonable time. In this communication we describe a plate replication procedure that can be used to screen large numbers of bacterial strains for the presence of lysogenic phages with a minimum of expended time and effort.

Briefly, the general procedure is as follows: representative young colonies (7 to 8 h old) of each strain to be tested are replicated by means of sterile toothpicks or by replica plating (velveteen template) to a master plate containing nutrients and agar and also to a second plate of the same medium supplemented with 0.01 or 1.0 µg of the inducing agent mitomycin C (MC) per ml. After overnight incubation of the plates to allow for growth and induction, the lysogenic cells produce partially lysed or, less frequently, mottled colonies on the MC plate. In some cases with higher concentrations of MC (5 or 10 µg/ml), no trace of the lysogenic colony remains. The last step of the procedure involves the replication from the MC plates to plates that have been seeded with potential indicator strains. An area of lysis on an indicator plate after incubation indicates sensitivity to a given lysogenic phage and also serves as a source of that phage. By means of this simple procedure, with as few as three plates we are able to ascertain the presence of phage (lysogenic or carrier) in a strain (by a comparison of the master and the MC plates), isolate the phages produced upon induction of that strain, and identify a suitable indicator for the phage. Because only a single colony of each strain is required to test for inducibility, we are able to screen as many as 35 different strains on a single master plate when replication by toothpick is used. We have demonstrated the efficacy of this procedure in trial runs with known lysogenic and carrier systems as well as with several strains suspected of harboring lysogenic phages. *Escherichia coli* K12 (λ) and *E. coli* C600 sensitive to λ were obtained from C. S. Buller; they were grown in TBM medium as described by Young and Sinsheimer (10). *Staphylococcus aureus* 8325 carrying phage P11 and P11-indicator strain *S. aureus* 8325-4 were received from R. P. Novick (7); 15 other strains of *S. aureus* (Table 1) previously uncharacterized or partially characterized with respect to lysogeny were obtained from P. A. Pattee (8, 9). All strains of *S. aureus* were grown in Difco brain heart infusion (8) supplemented with 400 µg of CaCl₂ per ml (2). *Micrococcus luteus* 15800 carrying phage N5 (3) and indicator strain *M. luteus* 15801 were obtained from W. E. Kloos; they were grown as described by Kloos and Schultes (5). Lawns to which MC-induced colonies were to be picked were prepared by the soft-agar overlay method (1) by using the media described above plus 1.5% agar as base and plus 0.6% agar for overlay. Indicator lawns for the velveteen template procedure (6) were prepared by spreading cells directly onto plates containing media plus 1.5% agar. All incubations were at 37 C. MC was sterilized by filtration and added to the autoclaved media at 45 C before pouring the plates.

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### Table 1. Demonstration of bacteriophages in strains of *S. aureus* by the plate induction procedure

| Induced strains | 8325-4 | 1 | Ps6 | 655 | 248 | W-26 | U9 | 655C | Ch-50 | 152 | 4965 | 112 | N-135 | C-72 | 113 | 8325 |
|-----------------|--------|---|-----|-----|-----|------|----|------|------|-----|------|-----|-------|------|-----|------|
| Ps6             |        |   |     |     |     |      |    |      |      |     |      |     |       |      |     |      |
| 655             | +      |   |     |     |     |      |    |      |      |     |      |     |       |      |     |      |
| 248             |        |   |     |     |     |      |    |      |      |     |      |     |       |      |     |      |
| W-26            |        |   |     |     |     |      |    |      |      |     |      |     |       |      |     |      |
| U9              |        |   |     |     |     |      |    |      |      |     |      |     |       |      |     |      |
| 655C            |        |   |     |     |     |      |    |      |      |     |      |     |       |      |     |      |
| Ch-50           |        |   |     |     |     |      |    |      |      |     |      |     |       |      |     |      |
| 152             |        |   |     |     |     |      |    |      |      |     |      |     |       |      |     |      |
| 4965            |        |   |     |     |     |      |    |      |      |     |      |     |       |      |     |      |
| 112             |        |   |     |     |     |      |    |      |      |     |      |     |       |      |     |      |
| N-135           |        |   |     |     |     |      |    |      |      |     |      |     |       |      |     |      |
| C-72            |        |   |     |     |     |      |    |      |      |     |      |     |       |      |     |      |
| 1               |        |   |     |     |     |      |    |      |      |     |      |     |       |      |     |      |
| 113             |        |   |     |     |     |      |    |      |      |     |      |     |       |      |     |      |
| 8325            |        |   |     |     |     |      |    |      |      |     |      |     |       |      |     |      |
| 8325-4          |        |   |     |     |     |      |    |      |      |     |      |     |       |      |     |      |

* Colonies were transferred by toothpick from master plates to plates containing 0.1 μg of MC/ml. After incubation of the MC plates to allow for growth and induction, the colonies (both visibly lysed and unlysed) were picked to plates seeded with indicator. Plaques were tested for the presence of infective particles by propagation on the homologous indicator. +, Plaque-containing infectious particles; -, absence of visible lysis.

Indicates lysis when colonies grown in the absence of MC were picked to indicator plates.

Figure 1 series A illustrates the results obtained by the velveteen template procedure by using *E. coli* K12 (λ) as the lysogenic strain and *E. coli* C600 as indicator. After 10 to 14 h of incubation, the colonies that appear on the MC plate have a more ragged and dispersed appearance (A2) when compared with the morphology of the uninduced colonies grown in the absence of MC (A1). Lysogenic cells that were transferred to MC plates by toothpick (Fig. 1 series B) yielded areas of sparse or almost no growth (B2) compared with those transferred to the non-MC plates (B1). When replicated by either procedure to lawns of indicator cells, each induced colony gave rise to an area of lysis. We have demonstrated by picking these plaques and propagation on C600 that they contain infectious particles. Because the velveteen template procedure very often gave smeared or indistinct plaques on indicator plates, all further experiments were carried out by using the toothpick method to make replicas.

*S. aureus* 8325 (7) and *M. luteus* 15800 (3), both known carrier strains, were tested for the presence of phage by the toothpick procedure, and the results were entirely equivalent to those obtained with *E. coli* K12 (λ), i.e., 100% of the colonies tested yielded plaques when picked to sensitive indicators. As a blind test of the validity of our procedure, 15 strains of *S. aureus*, the lysogenic status of which were unknown, were tested against each other. *S. aureus* 8325 and 8325-4 mentioned above were also tested in the same experiment. Transfer by toothpick from the MC to the indicator plates was done in parallel rather than series to avoid cross contamination of one indicator with another. Colonies grown in the absence of MC were also picked to indicator plates to check for spontaneous induction. The results are shown in Table 1. We have demonstrated that these plaques do indeed represent phage other than bacteriocinic activity by picking each plaque and propagating the particles on homologous indicator. Our finding that strain 655 harbors a phage that plaques on strain Ps 6 confirms a previous observation of P. Curtis (P. A. Pattee, personal communication). Five of the 11 *S. aureus* strains (N-135, 1, 4965, Ch-50 and 152) that did not produce plaques on any of the indicators were subjected to the classical method of induction in broth utilizing MC (0.1 and 1.0 μg/ml) in order to ensure that we were not getting false negatives by our plate procedure. The procedure was essentially the same as that described by Goldberg and Bryan (4); samples were harvested by centrifugation at 0.5-h intervals after MC treatment until lysis (monitored by OD at 600 nm) was complete. The lysates were tested (sintered glass filtrates) by spotting on seeded lawns (agar overlay). The results of these broth inductions were
producenumber of plaques on TBM broth without added induction. Lysates derived from non-induced cells of strain 1 produced a few plaques (approx. 7×10^4/ml) on strain 113; no plaque was obtained from the induced cells of strain 1. Apparently our failure to detect the phage of strain 1 by our plate procedure can be explained in terms of the extremely low yield of infectious particles. Lysates of strains N-135, 4865, and 152 produced clear zones on indicator strain 113; however, we were unable to propagate phage from these lysed areas, suggesting that they were the result of bacteriocin activity. Thus, there appears to be less interference from bacteriocins in the plate induction procedure compared with the usual broth procedure.

The plate method for the isolation of lysogenic phages should prove useful for those bacterial species such as N. gonorrhoeae for which no phages have yet been isolated and for which a phage-typing system would be especially valuable.

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