APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1977, p. 1007-1008
Vol. 33, No. 4

Printed in U.S.A.

Technique for Isolating Phage for *Azotobacter vinelandii*

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Received for publication 10 January 1977

An enrichment technique was developed whereby azotophage could readily be isolated after inoculation of soil sites with *Azotobacter vinelandii*.

During a search for a transducing phage for *Azotobacter vinelandii*, it became desirable to develop an efficient method of phage isolation. Previous attempts in this laboratory (T. Jones, unpublished data) have shown that azotophage are difficult to isolate, even though enrichment of naturally occurring *Azotobacter* in samples of soil, water, and sewage was performed by methods described by Duff and Wyss (2). Since selected species of bacteria are often isolated from soil through the use of enrichment media, we thought that an analogous technique utilizing cells of *A. vinelandii* might be useful for the isolation of azotophage. Thus, a specific site in the soil could be inoculated with the bacteria, and, hence, any preexisting azotophage should increase to an easily detectable level.

This paper describes the details of this enrichment technique and the results of a search for azotophage conducted during the summer. The sites selected for enrichment were high in decomposed organic material. Inoculum for each site was prepared by growing a 15-liter culture of *A. vinelandii* to a cell density of about 5×10^8 cells/ml in modified Burk N-free medium (3). After inoculation, each site was irrigated with 1 liter of 2% sucrose every other day. Soil sample extracts for phage assay were prepared by suspending 5.0 g of soil in 15 ml of phosphate buffer of the same composition as that in modified Burk medium. Soil particles were removed from the extract by centrifugation. The extract was filter-sterilized (45-μm-pore size membrane filter [Millipore]), and then 1 ml of the sterile soil extract was added to 19 ml of a culture of *A. vinelandii* containing about 2.5×10^7 cells/ml. The culture was allowed to incubate overnight with vigorous shaking at 30°C. Filter-sterilized samples (0.1 ml) of this culture were tested for the presence of phage by the soft agar overlay method (1). Purified phage lysates were obtained by confluent lysis on soft agar overlay plates.

The results of the enrichment technique with *A. vinelandii* strain OP are shown in Table 1. Phage were isolated from all inoculated sites. On the other hand, no phage were isolated from noninoculated sites, even when those sites were supplied with media and sucrose solutions. On the basis of plaque morphology, 21 different phage isolates were obtained. No phage were detected in any of the sites prior to inoculation with *Azotobacter* cells. The enrichment technique was repeated with *A. vinelandii* strain ATCC 12837 with essentially the same results. Eight isolates were obtained from eight new sites. No phage were detected in the preinoculation samples.

The possibility that our results were due to the induction of lysogenic phage, carried by the bacterial strains used for inoculation, appears to be unlikely since no phage could be detected after various times of ultraviolet light irradiation or mitomycin C treatment.

While we did not find a phage capable of transduction, the enrichment technique de-

TABLE 1. *Results of enrichment procedure for azotophage isolation*

Sample period (weeks)	Plaque-forming units/ml of enrichment culture					
	Noninoculated control	Site 1	Site 2	Site 3	Site 4	Site 5
Preinoculation	0	0	0	0	0	0
1	0	0	0	10	10	0
2	0	0	0	7.3×10^{10}	2.4×10^7	8.0×10^2
3	0	2.6×10^2	10	10^6	3.2×10^7	2.0×10^2
4	0	4.7×10^8	9.3×10^3	6.0×10^1	Not done	Not done

scribed above was effective in the isolation of azotophage. This method should be useful in other situations where phage are difficult to isolate by random sampling techniques.

This investigation was supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, by the Rockefeller Foundation, and by NSF grant BMS74-01134.

We thank Joyce Gordon, Brad Hersh, Walter Leps, and Robert Maier for their assistance in maintaining enrichment sites.

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