FACTORS AFFECTING THE PLAQUE-YIELD RESULTING FROM INFECTION OF E.COLI K12 PROTOPLASTS BY PHENOL EXTRACTS OF THE RNA-PHAGE ft5*

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Phage ft5** is related to the RNA-phages described by Loeb and Zinder (1) in its host specificity, composition, electronmicroscopic appearance and size (2). Its phenolextract (phenol-κ***) is inactivated by RNAase and not by DNAase (3).

Phage ft5 was used for isolation of genetic variants after incubation of phage suspensions with nitrous acid (4). To study the influence of different bacterial hosts on details of plaque morphology of such mutants, attempts were made to obtain ft5 yields also from E.coli K12 F⁻ strains. Therefore a method was adapted which is based on the infectivity of phenol-π when incubated with E.coli K12 protoplasts. While the manuscript was in preparation a publication was received by Fouace et al.(5), which described some of the variables of the plaque-yield in a phenol-π-protoplast system of FH_5-RNA-Phage. The present paper regards pertinent points concerning factors that affect the plaque-yield of a similar system using Phage ft5. Details will be published elsewhere.

Materials and Methods

Cells of E.coli K12 strains were grown in nutrient broth. Protoplast- (PPL) and T2 urea-π-techniques were essentially those de-

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*** This nomenclature (6) has been adopted as long as phenolextracts are not characterized in more detail.

scribed by Mahler and Fraser (6). Modifications are described below. Phenol-π was obtained from ft5 by adaptations of the method of Gierer and Schramm (7), when highly purified stocks were used, or by the method of Fraenkel-Conrat et al. (8), when raw lysates were used. The effeciency of protoplasting was determined by assay of colony formers or by control infections of PPL with T2 urea-π.

Results

1. Effect of temperature, time and phenol concentration on extraction. The standard method of extraction was as follows. One volume of raw lysate (titer $0.5\text{-}2\text{x}10^{12}$) was mixed with 1/2 volume of a bentonite* suspension (50 mg dry weight per ml in 0.01 M Na-acetate buffer, pH 6) and incubated at 37°C for 30 minutes. The bentonite was removed by centrifugation and an equal volume of bentonite suspension was added. All further manipulations were executed in the cold. To the total volume of the last mixture 1/4 volume of water-saturated phenol was added. After 5 minutes of extraction with shaking the layers were separated by centrifugation and processed as usual (7,8). Plaque-yields obtained by deviations from this method are shown in table 1.

Table 1

EFFECT OF VARIATION OF EXTRACTION PROCEDURE ON PLAQUE YIELDS

Normalized data from separate experiments.

experimental conditions		Plaque-yield
Temperature of extraction	0°C	1140
	25°C	172
	50°C	14
	80°C	0
Time of extraction	5 min	1140
	10 min	630
Proportion of phenol in extraction mixture	1/8 vol	840
	1/4 vol	1140
	1/2 vol	320

*Bentonite was kindly furnished by the Geisenheimer Kaolinwerke, Erbsloeh, Geisenheim/Rhein.

2. Effect of time, temperature, and pH on protoplasting

Cells were washed after harvesting with TRIS - buffer pH 8 or pH 9, resuspended in 0.5M sucrose at the desired titer, and warmed to 25 or 37°C. Lysozyme and versene were added as usual (6). The protoplasts resulting after various times of incubation were infected with phenol-T and plated after 10 minutes of further incubation. The plaque-yields are shown in table 2.

Table 2

EFFECT OF TIME, TEMPERATURE, pH ON PROTOPLASTING EXPRESSED IN PLAQUE-YIELDS

Normalized data from separate experiments.

experimental conditions			Plaques
Tris buffer wash		pH 8	1000
		pH 9	450
Cell concentration in sucrose		$1x10^{10}$	1000
		$5x10^{9}$	400
Temperature and time of incubation for protoplasting	37°C	10 min	730
		30 min	1000
		60 min	810
		90 min	400
	25°C	10 min	22
		30 min	130
		60 min	270
		90 min	325

3. Effects of the metabolic state of cells used as source for protoplasts. Cells from an overnight-exponential culture (10) were diluted to 1/10 of the desired final concentration and permitted to grow with aeration. Protoplasts were prepared from cells of various densities of growth and infected with T. The plaque-yields were highest, if cells were used when grown to $2\text{-}4x10^{8}$. If grown to $1\text{-}3x10^{7}$, yields were reduced to 15-20%, if grown to $1x10^{9}$ and higher, yields were reduced to 10% or less.

4. Effect of bovine serum albumin on protoplasts. The stabilizing effect of bovine serum albumin (BSA) on protoplasts has been observed frequently (5,6,11,12) as well as its inhibitory action on the production of plaques resulting from infection of PPL with phenol-π (5). Highest yields were obtained if BSA was omitted (table 3). Protoplasts without BSA were sufficiently stable for manipulations, such as dilution in 0,25 M sucrose containing 1/2 the usual concentration of nutrient broth.

Table 3.

EFFECT OF BSA ON PLAQUE YIELDS AFTER INFECTION OF PPL WITH s.

Concentration of BSA in PPL suspension	Plaque yield after PPL infection with	
	ft5 phenol-π	T2 urea-π
1.0 %	160	80
0.2 %	260	100
0.0 %	680	800

5. Effect of density of cells used for protoplasting.

Since only high concentrations of cells used for protoplasting give high plaque yields, depending on the efficiency of protoplasting, the titer of colony formers left in the PPL-π infection mixture is of the order of 10^8 or more. These cells only poorly support the formation of plaques. They instead interfere with it. This is demonstrated by a drop in plaque-yield after adding to infected protoplasts a sample of cells from an exponential culture grown to $4x10^8$ (the cell concentration and age which support maximum development of plaques of ft5 (13)) and plating subsequently. Dilution of the PPL-π infection mixture before plating therefore results in an relative increase in plaque-yield (table 4).

Table 4.

EFFECT OF DILUTION OF THE PPL- INFECTION MIXTURE ON PLAQUE-YIELD

Efficiency of protoplasting was 85 % as determined by assay of colony formers

Dilution	Plaque-yield		
	obtained	expected if standard is dilution	
		0	1/20
0	640	640	2560
1/2	800	320	1280
1/5	480	128	640
1/10	360	64	320
1/20	160	32	160

Optimal conditions permit the recovery of plaques of ft5 of the order of 10^{-8}, if plaque-yields resulting from infections of PPL with ft5-π are compared with the plaque-forming capacity of the lysate used for phenol extraction. This is more than the recoveries reported for example for phage FH_5 (5), which was between $5x10^{-10}$ and $5x10^{-9}$. Work on further improvements of recovery, which is desirable for a number of projects in progress, is continued.

References

1. Loeb, T., and Zinder, N. D., Proc. Natl. Acad. Sci., U.S., 47, 282 (1961)
2. Hoffmann-Berling, H., unpublished
3. Knolle, P., in preparation
4. Knolle, P., and Kaudewitz, F., in preparation
5. Fouace, J., and Huppert, J., Compt. rend. 254, 4387 (1962)
6. Mahler, H. R., and Fraser, D., Virology 8, 401 (1959)
7. Gierer, A., and Schramm, G., Z. Naturforsch. 11b, 138 (1956)
8. Fraenkel-Conrat, H., Singer, B., and Tsugita, A., Virology 14, 54 (1961)
9. Adams, M. H., Bacteriophages, Interscience Publishers, New York-London, 1959
10. Knolle, P., in press
11. Spizizen, J., in: Methods in Enzymology V, 122, Academic Press, New York-London, 1962
12. Huppert, J., Wahl, R., and Emerique-Blum, L., Biochim. Biophys. Acta 55, 182 (1964)
13. Knolle, P., in preparation