INFECTION OF ESCHERICHIA COLI K-12 WITH RNA OF ENCEPHALOMYOCARDITIS VIRUS

Renana Ben-Gurion and Yael Ginzburg-Tietz
Israel Institute for Biological Research, Ness-Ziona, Israel

Received November 16, 1964

A method for introducing DNA molecules into E. coli strains, was described by Kaiser and Hogness (1960). This method was used by us to introduce infectious RNA of Encephalomyocarditis (EMC) virus into E. coli strain W 3693 (λ).

Infectious RNA of EMC virus was recovered by Huppert and Sanders (1958) and was found by them to be able to infect mouse ascites cells maintained in vitro. We prepared infective RNA by the method described by Bellett et al (1962) who used phenol extraction in the presence of bentonite. The infective RNA was isolated from the culture fluid into which Krebs-2-ascites cells had released virus. (Ginzburg, Kaufmann and Traub, 1964). A preparation containing 3×10^5 infectious units per 100 gamma RNA, was thus obtained. (One infectious unit is equivalent to the smallest quantity of material injected intra - cerebrally, that kills a suckling mouse). This preparation was free from whole viral particles and was thus sensitive to RNAse. (The infectivity of the RNA preparation was abolished in 15 min at 37°C by 50 µg/ml.)

Infection of the bacteria with the RNA preparation was found to proceed best, using the following procedures : Strain W3693 (λ), an uracilless auxotroph, was grown in minimal medium (Ben-Gurion 1963 a), until it reached the late log phase, when the number of bacteria/ml were of the order 3×10^8 to 6×10^8 cells/ml. The suspension of these bacteria was then kept over night at 4°C. Another suspension of early log phase bacteria (5×10^7 to 1×10^8 cells/ml) was prepared the next morning. These two suspensions were irradiated with ultra-violet light for one minute (Ben-Gurion and Hertman,(195 After the irradiation of these two suspensions, the bacteria were thoroughly washed on milipore filters with $MgSO_4$ 0.02 M, and concentrated into suspensions of cells containing 2 to 3×10^9 bacteria/ml each. The two bacterial suspensions were then mixed in equal proportions, and 0.2 ml of infectious RNA solution containing 3×10^5 infectious RNA particles/ was added to 0.1 ml of the bacteria. To this mixture 0.2 ml of 1×10^{10}/ml phage λ particles

was added from a stock containing 3-5 x 10^{10} wild type λ obtained by u.v. irradiation of strain 169 (λ) (Ben-Gurion, 1963 b). This mixture was left at room temperature for 10 minutes, then incubated for an additional 10 minutes at 37°C. After this time, the infected bacterial suspension was divided into two : one sample was diluted into minimal medium without glucose, and the other sample was diluted into minimal medium lacking both glucose and uracil. Cysteine at a concentration of 2.5 μg/ml was added to both samples which were then incubated on a shaker at 37°C for 12 minutes. Glucose was then added to both samples, and the bacteria were further incubated at 37°C. This treatment (with cysteine and without glucose for 12 minutes) had the purpose to prevent the induction of these lysogenic bacteria by the irradiation (Ben-Gurion 1963 a, and Ben-Gurion in press). The bacteria thus treated, were incubated at 37°C. After the infected bacteria had been incubated 5 hours, they were held at 4°C over night, and the incubation was resumed the next morning. At various intervals during incubation samples were withdrawn and subjected to 15 minutes sonication which reduced the number of viable bacteria to less then 10 %.

For this purpose a model DF-101 Raytheon Sonic Oscillator was used at 10Kc/sec , cooled by circulating ice water through the jacket of the sonicating chamber. The sonicates

TABLE 1

The lethal effect of sonicates at various incubation times.

incubation time of (hours)	additional treatment of the sonicates	number of dead mice/number of injected mice					
		bacteria incubated					
		with uracil			without uracil		
		dilution of the orginal RNA solution					
		1:70	1:700	1:1400	1:70	1:700	1:1400
0	---	2/8	0/8				
2	---	0/8	0/8		0/8	0/8	
4	---	0/8	0/8		0/8	0/8	
6	---	0/8	0/8		0/8	0/8	
8	---	4/8	3/8		6/8	8/8	
8	+RNAse			0/8			5/8
10	---	8/8	8/8		3/8	0/8	
10	+RNAse			4/8			0/8

were injected (0.03 ml per mouse) into the brains of suckling mice, at various dilutions.

The injections were carried out with : a) crude sonicates of infected bacteria, b) with RNAase treated sonicates (50 µg RNAase/1 ml for 15 minutes at 37°C) c) with sonicates incubated for 30 minutes at room temperature with rabbit anti-EMC serum ;

TABLE 2

The lethal effects of sonicates at various incubation times, without uracil treated with anti-EMC serum

incubation time (hours)	additional treatment of sonicates	number of dead mice/number of injected mice	
		dilution of original RNA solution	
		1:50	1:500
0	None	1/8	0/8
0	+RNAase	0/8	0/8
0	+anti-EMC	0/8	0/8
0	+Normal serum	0/8	0/8
3	None	0/8	0/8
3	+RNAase	0/8	0/8
3	+anti-EMC serum	0/8	0/8
3	+Normal serum	0/8	0/8
6	None	7/8	0/8
6	+RNAase	6/8	0/8
6	anti-EMC	0/8	0/8
6	+Normal serum	6/8	0/8
8	None	8/8	4/8
8	+RNAase	8/8	3/8
8	+anti EMC	0/8	0/8
8	+ Normal serum	7/8	3/8
10	None	2/8	0/8
10	+RNAase	1/8	0/8
10	+anti EMC	0/8	0/8
10	Normal serum	0/8	0/8

a treatment that neutralized completely the infectivity of a suspension containing 10^4 EMC viral particles/ml. d) with sonicates incubated for 30 minutes at room temperature with normal rabbit serum ; a treatment that did not affect the infectivity of EMC particles.
Mice injected, as controls, with bacteria which had not been infected with EMC RNA but otherwise treated in the same way and also incubated for the same period of time, did not die.

When the same experiments as those described in Tables 1 and 2, were repeated without helper phage, the 6, 8 and 10 hours sonicates had no lethal effect .

Infection was thus found to occur only if the bacteria exposed to EMC RNA were simultaneously infected with phage λ. Further studies are necessary to determine whether the conditions of infection carried out in this work are optimal, or whether different conditions will give better yield of virus particles. Preliminary experiments, in which "lysis from without" of the infected bacteria, with phage T2, was carried out in order to release the virus from the bacteria, yielded positive results, but the titer of the virus released was smaller than when the release was carried out by sonication of the bacteria.

From the results summarized in this report, we conclude that infectious RNA EMC virus can infect E. coli K-12 (strain W3693 λ) and form in the infected bacteria complete virus particles. That the material produced in the infected bacteria is EMC virus, was shown by : a) its resistance to RNAase, b) its sensitivity towards anti EMC antibodies. The complete virus was produced in the infected bacteria also when uracil was not added to the medium. This does not mean that RNA synthesis is not involved here, since it was shown by Nakada and Smith (1962), that uracilless strains of E. coli produce messenger and transfer RNA in the absence of added uracil.

The authors would like to thank Dr. R. Oren for the gift of anti EMC serum, as well as Miss E. Burstein and Miss H. Schwammbaum, for excellent technical assistance.

References

Bellett, A.J.D. Burness, A.T.H. and Sanders F.K., Nature, 195 , 874 (1962).
Ben-Gurion, R., Biochem. Biophys. Res. Commun., 11 , 5, 399 (1963 a).
Ben-Gurion, R., Virology, 20 , 288 (1963 b).
Ben-Gurion, R., J. Mol. Biol. (in press).
Ben-Gurion, R. and Hertman, I., J. Gen. Microbiol 19 , 289, (1958).
Ginzburg, Y. Kaufmann, E., and Traub, A., Exp. cell Research, 34 , 2, 384 (1964).
Huppert, I. and Sanders, F.K., Nature, 182 , 515 (1958).
Kaiser, A.D. & Hogness, D.S.J., Mol. Biol., 2 , 392 (1960).
Nakada, D. & Smith, I., Biochem. Biophys. Acta., 61 , 414 (1962).