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LYSOGENY BY MS2 PHAGE. ANALYSIS OF A RECOMBINANT PLASMID CONTAINING MS2 RNA-LIKE SEQUENCE

pL34 recombinant plasmid coding MS2-like RNA synthesis in plasmid-containing cells has been studied using physical mapping and blot-hybridisation with P^{32}-labelled pMS27 plasmid fragments including a DNA copy of MS2 phage RNA. The authors have determined the size of a fragment containing MS2-like sequence and its localisation inside the hybrid plasmid.

Introduction. Dispite of RNA-containing phages being among the best studied biosystems, some of their properties, especially phage-host interactions, have not been completely investigated. One of the problems is a cause of multiple resistant cells development in *E. coli* cultures infected by RNA-containing phages in the conditions not favorable for quick cell division. In some previous papers concerning RNA-containing phages biology this phenomenon has been discussed from the point of view of possible preexisting mutants selection. Nevertheless, such an interpretation does not seem to be completely convincing because of large scale phage resistance development. In our previous paper [1] we have shown that more than 1 % of infected bacterium offspring receives *MS2*-resistance marker which may not be due to pre-existing mutants selection. We interpret such a fact as a non-direct proof of direct interactions between phage genome and cell host DNA; out interpretation has been confirmed by non-stability of primary *MS2*-induced mutations and derivative forms segregation from these ones [2]. In order to prove that these mutants properties are caused not by a persistent *MS2* infection but due to phage genome integration into the host cell chromosome we have created a genetic library of *EcoRI* fragments of a segregant obtained by us — a lysing *E. coli* AB 259 Hfr 3000 mutant [3]. A total RNA preparation isolated from plasmid-forming cells has been hybridised both with *pMS27* DNA containing *MS2* phage cDNA and with a part of this cDNA without vector sequence. The results obtained prove phage specific RNA to be transcripted from chromosomal DNA of the *MS2*-induced *E. coli* mutant; so we have really demonstrated that *MS2* phage is able to establish true lysogeny as a state based on physical integration of phage and cellular DNA's. In the present paper we present our first data on our study of cloned host cell DNA fragment having the goal to determine how large is *MS2*-specific region and which is its localisation on the genetic map of our recombinant plasmid; we aim also to find there some recognition sites for several popular restrictive enzymes.

Materials and methods. P l a s m i d s. Recombinant plasmid *pL34* has been constructed by us using non-replicative *Ap*-fragment of *pCV16* plasmid and described in the previous paper [3] as *pL26* plasmid. A strain containing *pCV16* plasmid [4] has been received from the Institute of Biochemistry and Physiology of Micro-organisms (Russian Academy of Sciences, Pushtchino-na-Okie, Moscow district, Russian Federation). A strain carrying *pMS27* plasmid [5, 6] is a friendly present of Dr R. Devos (Gent University, Belgium).

Nutrient media. We have mostly used 0.6 % and 1.2 % amino peptide (AP) containing agar and AP-containing broth. Plasmid isolation, restriction analysis, and blot-hybridisation have been perfected according to [7]; physical mapping of plasmid DNA sequences has been made with mutual and single digestion approaches [8].

Results and discussion. To determine the size of a recombinant DNA plasmid fragment carrying *MS2*-like sequence and its localisation in the

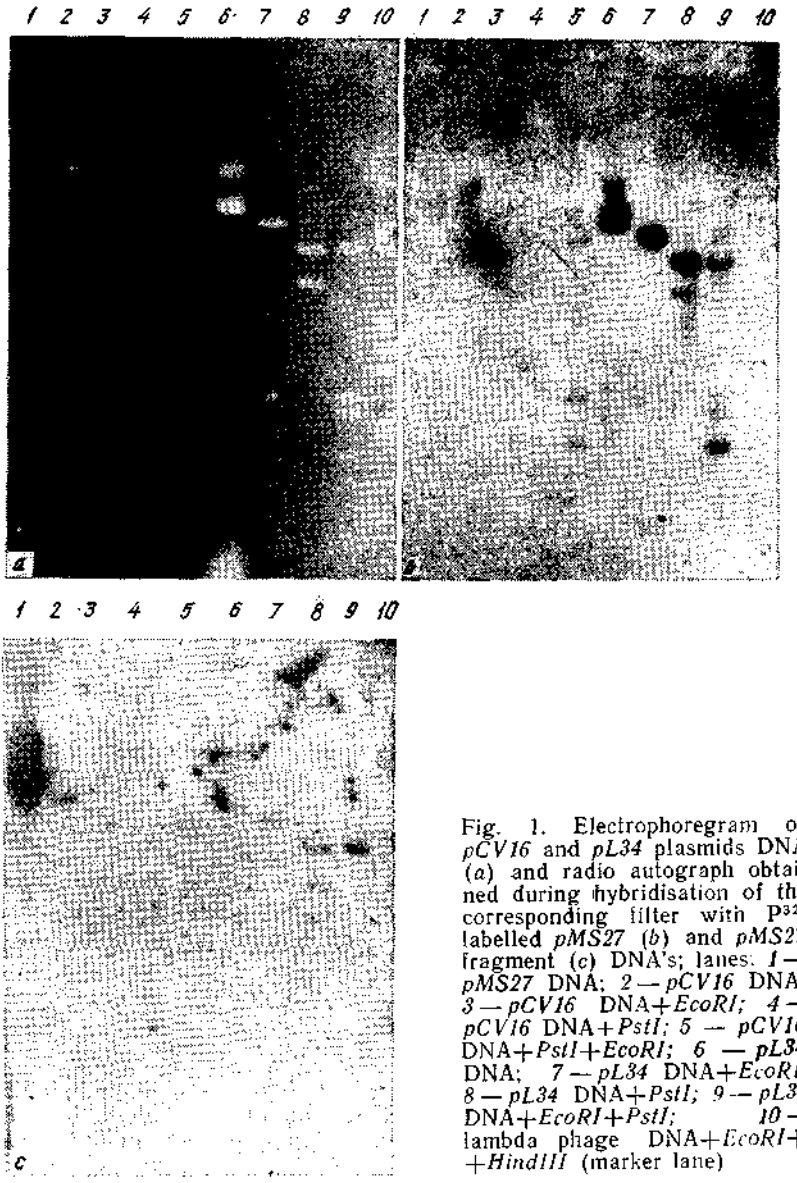

Fig. 1. Electrophoregram of *pCV16* and *pL34* plasmids DNA (*a*) and radio autograph obtained during hybridisation of the corresponding filter with P^{32}-labelled *pMS27* (*b*) and *pMS27* fragment (*c*) DNA's; lanes: *1* — *pMS27* DNA; *2* — *pCV16* DNA; *3* — *pCV16* DNA+*EcoRI*; *4* — *pCV16* DNA+*PstI*; *5* — *pCV16* DNA+*PstI*+*EcoRI*; *6* — *pL34* DNA; *7* — *pL34* DNA+*EcoRI*; *8* — *pL34* DNA+*PstI*; *9* — *pL34* DNA+*EcoRI*+*PstI*; *10* — lambda phage DNA+*EcoRI*+ +*HindIII* (marker lane)

recombinant plasmid we have used physical mapping and blot-hybridisation. Our probes were both the whole *pMS27* plasmid containing *MS2* cDNA and a fragment of this DNA copy without vector sequence. The results of an experiment with *EcoRI* and *PstI* treatment of *pL34* and *pCV16* plasmids are shown in the Fig. 1, *a*.

It is clear from the Fig. 1, *b* that the labelled *pMS27* having been used as a probe gives hybridisation with all the fragments of both plasmids (*pCV16* and *pL34*). It may be due to *pCV16* origin, i. e. to the presence of some homologies with *pBR322* sequences in the vector part of *pMS27*. In the next blot-hybridisation experiment (see Fig. 1, *c*) where

cDNA copies of phage *MS2* RNA have been used hybridisation lines correspond to *pCV16* fragments of the size 5.1 kbs and to *pL34* fragments as large as 5.1 and 4.8 kbs in single and double treatments. The smaller *pCV16* fragments (their sizes are about 1.1 and 0.7 kbs) corresponding to Ap-fragment of the total size 1.8 kbs do not hybridise with *pMS27* fragment, i. e. a region of the recombinant plasmid able to hybridise with *pMS27* fragment is not a part of *pCV16* plasmid.

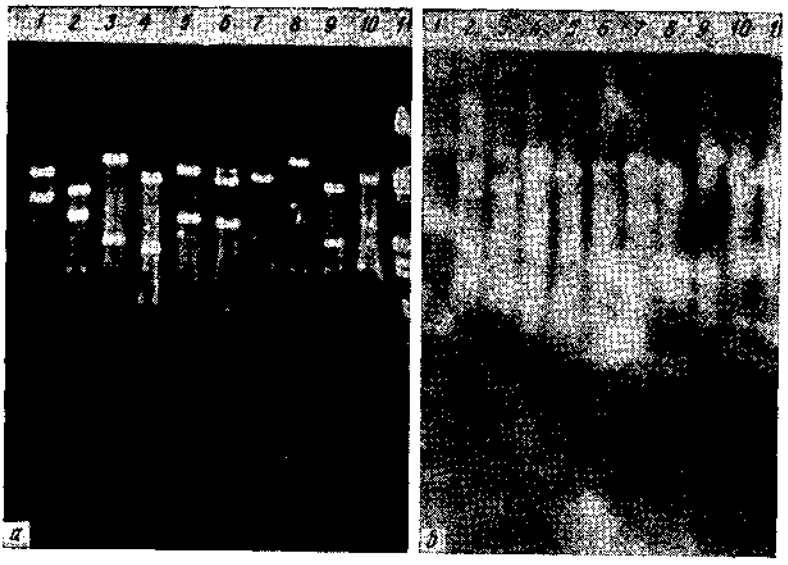

Fig. 2. Electrophoregram of *pL34* plasmid DNA (*a*) and radio autograph obtained during hybridisation of the corresponding filter with P^{32}-labelled *pMS27* fragment DNA (*b*); lanes: *1* — *pL34* DNA+*PstI*; *2* — *pL34* DNA+*PstI*+*BamHI*; *3* — *pL34* DNA+*BamHI*; *4* — *pL34* DNA+*BamHI*+*HindIII*; *5* — *pL34* DNA+*HindIII*; *6* — *pL34* DNA+*HindIII*+*PstI*; *7* — *pL34* DNA+*PstI*+*EcoRI*; *8* — *pL34* DNA+*EcoRI*; *9* — *pL34* DNA+*EcoRI*+*BamHI*; *10* — *pL34* DNA+*EcoRI*+*HindIII*; *11* — lambda phage DNA+*EcoRI*+*HindIII* (marker lane)

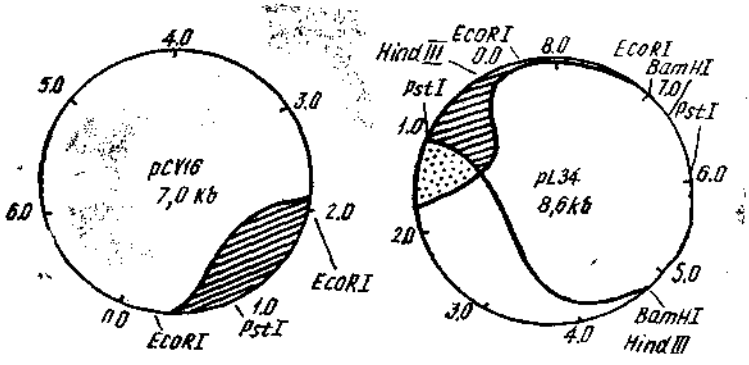

Fig. 3. Physical maps of *pCV16* and *pL34* plasmids. A shaded part corresponds to the fragment including ampicilline resistance gene, an unshaded and wider one corresponds to a fragment containing *MS2*-like sequence

Our data obtained in the experiments of recombinant and vector plasmids hybridisation with *pMS27* plasmid carrying *MS2* cDNA and with this cDNA copy permit us to conclude that the sequence homological to *MS2* RNA is situated in the large *pL34* fragment (5.1 kbs) limited by *PstI* sites. The hybridisation observed with *pCV16* DNA is thought to be most probably due to random short sequences, *pCV16* DNA being non-hybrisidable both with *MS2* RNA and also total RNA isolated from a strain containing a recombinant plasmid and being able to hybridise both with labelled *pMS27* plasmid and with its fragment [3]. These data suggest that on the contrary to recombinant plasmid *pCV16* plasmid con-

tains no sequence coding *MS2*-like RNA synthesis and forming hybrids with such RNA. So the hybridisation observed in *pCV16* experiments may be explained by a presence of a random sequence or of a sequence with the biological function analogue to lysogenic phage integration site in host cell chromosome. Electrophoregrams of the recombinant plasmid after *PstI*, *BamHI*, *HindIII*, and *EcoRI* restrictive digests as well as blot-hybridisation of fragments obtained with *pMS27* fragment are demonstrated in the Fig. 2.

Our results lead to the conclusion that *MS2*-like sequence in most probably localised inside the fragment as large as 3.9 kbs limited by *PstI* and *BamHI* cleavage sites. The localisation of this fragment is shown on the recombinant plasmid map given in the Fig. 3.

Fig. 4. Electrophoregram of *pL34* plasmid with corresponding lanes: *1* — *pL34* DNA+*PstI*+*BamHI*; *2* — *pL34* DNA+*PstI*+*BamHI*+*PvuII*; *3* — *pL34* DNA+*PstI*+*BamHI*+*BglII*; *4* — *pL34* DNA+*PstI*+*BamHI*+*SalI*; *5* — lambda phage DNA+*EcoRI*+*HindIII* (marker lane)

The undoubtful hybridisation of this fragment with a fragment of *MS2* cDNA fragment and almost total coincidence of its size (3.9 kbs) with *MS2* RNA size (3.569 kbs) [9] permit to think that *MS2*-like sequence integrated into *pL34* recombinant plasmid is localised inside this fragment. It should be noted, however, that this region contains no *EcoRI* and *SalI* sites detected inside *MS2* cDNA [10]. A *SalI* site situated inside of *PstI-BamHI* fragment (see Fig. 4) is localised not far from one of its termini corresponding in its localisation to none of two *SalI* sites detected inside DNA copy of the phage RNA [10]. So the region of recombinant plasmid being correspondent to *MS2* sequence according to hybridisation experiments is probably a fragment of the structure more complex than a double-stranded one. Such a conclusion is not contradictory to our previous results demonstrating that labelled recombinant plasmid DNA fails to hybridise with *MS2* RNA [3]. The absence of recombinant plasmid DNA and *MS2* RNA hybridisation accompanied by *MS2*-like RNA synthesis in plasmid-containing cells may be most probably explained by a hypothesis that this plasmid contains a plus-chain of *MS2* RNA-like DNA or even *MS2* RNA and has a three-stranded structure in the fragment of the size about 3.9 kbs. Our point of view is the last version is also probable because of being able to explain our negative results obtained when we have attempted to detect some restrictive sites inside *pL34* plasmid *PstI-BamHI* fragment with size of 3.9 kb, these ones being undoubtfully present in the DNA copy of *MS2* RNA.

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ЛІЗОГЕНІЯ У ФАГА MS2. АНАЛІЗ РЕКОМБІНАНТНОІ ПЛАЗМІДИ, ЩО МІСТИТЬ MS2 РНК-ПОДІБНУ ПОСЛІДОВНІСТЬ

Резюме

Рекомбінантну плазміду *pL34*, що кодує синтез *MS2*-подібної РНК у плазмідовмісних клітинах, вивчали методом фізичного картування та блот-гібридизації. Як зонд використовували фрагмент плазміди *pMS27*, що містить ДНК-копію РНК фага *MS2*. Було визначено розміри фрагменту, який включає *MS2* РНК-подібну послідовність, та його локалізацію в гібридній плазміді.

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