INHERITANCE AND PHENOTYPIC EXPRESSION OF PLASMIDS CODING EcoRI RESTRICTION ENDONUCLEASE IN *Vibrio cholerae* CELLS

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Restriction endonucleases are widely used nowadays to clone DNA fragments of different origin with the aid of vector molecules of plasmids and bacteriophages [8]. The process of DNA recombination, in which restriction endonucleases take part, also takes place *in vitro* [1, 7]. The obtaining of hybrid molecules *in vivo*, due to the action of restriction endonucleases can in many cases facilitate the task of cloning foreign DNA in bacterial cells. For instance, by means of a technique based on completion of a recA-independent recombination process, EcoRI-dependent cloning of DNA has been carried out in *Escherichia coli* cells *in vivo*, so that it was possible to obtain plasmids carrying $recB^+C^+$ genes or enterotoxin Ent genes [4].

Reproduction of this process in *Vibrio cholerae* opens up a new approach to the obtaining of recombinant plasmids carrying genes of *V. cholerae* and, in particular, genes responsible for the leading pathogenetic sign of *V. cholerae*, namely toxin formation. For this purpose it was necessary to transfer into *V. cholerae* cells a plasmid coding restriction endonuclease EcoRI, to preserve it in *V. cholerae* cells, and to express EcoRI activity in them.

The aim of this investigation was to construct such a plasmid and to investigate the phenotypic expression of its genetic determinants in *V. cholerae* cells.

EXPERIMENTAL METHOD

Strains of *E. coli* K-12 GA9 (trp^-), P3478 (polA1 am $thyA^-Str^S$) and GA87 (leu recBC sbcB leu^-proA2 $his^-argE^-thy^-Str^R$), plasmids R245 (Tra^+Tc R·M·EcoRII IncN), pSA1 (Tra^+Ap Sm Su Cm Cel R·M·EcoRi fi^+), strain *V. cholerae* RV31 ($arg^-ilv^-his^-Str^R$) were obtained from the collection of the N. F. Gamaleya Research Institute of Epidemiology and Microbiology; bacteriophages PIvir, λ, and CPT1 also were used. Minimal medium A, L-broth, and solid nutrient media prepared on their basis, were used.

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TABLE 1. Efficiency of Seeding Bacteriophages λ and PI on *E. coli* Strains GA9 and GA9/pSA1002

Modification	Seeding efficiency of λ		Seeding efficiency of PI	
	To GA9	To GA9/pSA1002	To GA9	To GA9/pSA1002
GA9	1	$4 \cdot 10^{-4}$	1	$1{,}5 \cdot 10^{-6}$
GA9/pSA1002	1	0,6	1	0,7

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TABLE 2. Transduction of Chromosomal Markers by Bacteriophages PI (modified by R. M. EcoRI and unmodified) from *E. coli* Strain GA9 into *E. coli* Strains GA87 and GA87/pSA1002

Strain of recipient	Transducing phage	Number of transductants per dish			
		His^+	Pro^+	Leu^+	Arg^+
GA87	PI·GA9	698	1132	4328	2180
	pI·GA9 pSA1002	1837	1414	3440	1760
GA87/pSA1002	PI·GA9	48	2	1389	406
	PI·GA9/pSA1002	153	179	704	54

TABLE 3. Efficiency of Seeding Phage CPT1 on Strains RV31 and RV31/pSA1002

Modification	Seeding efficiency	
	To RV31	To RV31/pSA1002
RV31	1	10^{-4}
RV31/pSA1002	1	0,8

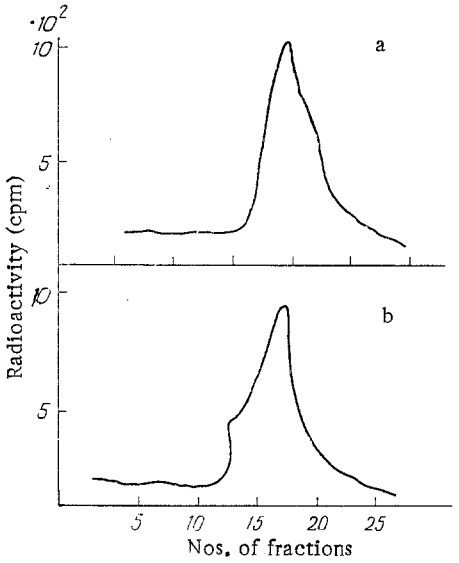

Fig. 1. Centrifugation of clarified lysates of *E. coli* strains P3478/pSA1002 (a) and GA87/pSA1002 (b) in 15-50% neutral sucrose gradient. Abscissa, nos. of fractions; ordinate, radioactivity (cpm).

Transduction with bacteriophage PIvir and conjugation transfer of plasmids between *E. coli* cells were carried out by the standard method [3]. Conjugation transfer of plasmids into *V. cholerae* cells was done on filters [5].

The results of three to five experiments are given in Tables 1-3; their level of significance ($P \leqslant 95.5$) was determined by the HP86 statistical program.

EXPERIMENTAL RESULTS

Plasmid genes coding the system of EcoRI restriction and modification are associated with colicinogenicity factor ColE1 [9]. Plasmids carrying R.M.EcoRI genes cannot undergo stable inheritance in strains of *E. coli* with polA1 or recBCsbcB mutations [6]. They can-

not be transmitted into *V. cholerae* cells. To construct plasmids containing EcoRI genes and transmitted into *V. cholerae*, strains containing plasmid pSA1, not transferred into *V. cholerae*, and plasmid R245, transferred into *V. cholerae*, were crossed. As a result, a series of recombinant plasmids was obtained, from which it was possible to transfer only the plasmid named pSA1002 into *V. cholerae* cells.

Plasmid pSA1002 can exist in *E. coli* strains P3478 and GA87 in the nonintegrated state, as is proved by the obtaining of clearly defined peaks of plasmid DNA, detectable by sedimentation analysis of DNA of strains carrying plasmid pSA1002 in a 15-50% neutral sucrose gradient (Fig. 1).

Analysis of the genetic properties of plasmid pSA1002 showed that it codes resistance to ampicillin and tetracycline, and ColE1 immunity, but does not determine production of colicin ColE1 by the cells. Strains carrying pSA1002 carry out restriction of phages λ and PI, and also modify them (Table 1), which suggests that R·M·EcoRI genes are present in the composition of the plasmid. Experiments also were carried out to detect specificy of action of restriction endonuclease EcoRI, coded by plasmid pSA1002, *in vivo*. For this purpose, transformation of EcoRI-modified and unmodified chromosomal homologous DNA [6] and transduction: phage PIvir modified by the R·M·EcoRI system and unmodified phage PIvir (Table 2), were carried out into strain GA87. The results show that during transduction with unmodified phage the frequency of formation of Pro^+ recombinants was reduced by more than 100 times compared with the frequency of formation of these recombinants in analogous experiments with modified phage. The frequency of formation of Leu^+ and Arg^+ transductants when unmodified phage was used, on the other hand, was increased by 2-3 and 5-10 times. The frequency of transduction with respect to the His^+ marker was unchanged. The results agree largely with data for the specificity of incision of transforming markers of *E. coli in vitro* by restriction endonuclease EcoRI [2].

The facts described above suggest that plasmid pSA1002 confers the phenotype Ap TcR·M·EcoRI Col_{imm} on the cells.

It was shown by crossing strains GA9/pSA1002 and RV31 that plasmid pSA1002 is transferred into *V. cholerae* cells from *E. coli* with a frequency of 10^{-4} transconjugants per donor's cell.

Determinants of antibiotic resistance to ampicillin and tetracycline are expressed in *V. cholerae* cells. Immunity to the action of colicin ColE1 could not be tested in *V. cholerae*, since the *V. cholerae* cells were found to be naturally immune to colicin ColE1. However, preservation of the Col_{imm} determinant in the composition of plasmid pSA1002, contained in *V. cholerae* cells, was demonstrated by transfer back from *V. cholerae* into *E. coli*. During this transfer, markers of antibiotic resistance and of R.M.EcoRI activity also were preserved. Thus plasmid pSA1002 undergoes stable inheritance in *V. cholerae*.

To investigate expression of R·M·EcoRI genes in *V. cholerae* cells, we used plasmid RP4::Mucts62 [5], which was constructed in our laboratory and which has several incision sites for EcoRI *in vitro*, and also a transducing bacteriophage CPT1, generously provided for us by Ogg (USA). In experiments with RP4::Mucts62 the frequency on conjugation transfer into strain RV31/pSA1002 was reduced by more than 100 times ($2 \cdot 10^{-4}$ transconjugants per donor's cell) compared with the frequency of transmission into the recipient strain RV31 without plasmid (10^{-6} transconjugants per donor's cell). Phage CPT1 was restricted and modified by the R·M·EcoRI system in strain RV31, which carries plasmid pSA1002 (Table 3).

Expression of activity of restriction endonuclease EcoRI in *V. cholerae* can thus be taken as proven, which means that plasmid pSA1002 can be used to reproduce the process of EcoRI-dependent recombination in order to clone chromosomal genes of *V. cholerae in vivo*.

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