OBTAINING *Vibrio cholerae* R'his PLASMIDS

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Much progress has now been made in the genetic study of cholera vibrios, and as a result the construction of active donors of genetic material and, in particular, of Tfr- and Hfr-strains, has been facilitated [3, 7]. However, no data could be found in the literature on the method of obtaining R'-strains of cholera vibrios.

Previous investigations showed that plasmid RP4::Mu cts 62 mobilizes the chromosomal genes of *Vibrio cholerae* for transmission [2]. A marked increase in the mobilizing activity of the plasmid was observed at a semipermissive temperature (37°C) for phage Mu cts 62. Analysis of transconjugants showed that clones selected with respect to chromosomal markers do not carry plasmid markers. This suggested that, under the conditions of functioning recombination systems of *V. cholerae* clones acquiring the donor's chromosomal markers are evidently formed on account of chromosomal recombinants. Meanwhile data have been obtained to show that under certain conditions transmission and preservation of the chromosomal genes of *V. cholerae* can be obtained in the composition of plasmid RP4::Mu cts 62, i.e., that strains of R' type can be obtained. These conditions were provided by the property of the donor of the genetic material. Conjecturally, this property was the presence of homology in the plasmid and in the chromosome arising on account of phage Mu, incorporated into the chromosome and a component of the plasmid.

This paper describes data confirming this hypothesis and indicating the possibility that R'-strains of *V. cholerae* effectively transmitting chromosomal genes can be obtained.

EXPERIMENTAL METHOD

A list of the strains used in the work is given in Table 1.

Phage Mu and its ts-mutant Mu cts 62 were obtained from M. M. Howe (USA) [6]. The nutrient medium used in the work and also the techniques of thermoinduction of prophage, conjugation transmission of plasmids, and mobilization of chromosomal genes with the aid of these plasmids were described previously [1, 2, 6].

EXPERIMENTAL RESULTS

The original strain used to obtain R'-donors was strain 569B. This strain grows poorly at 42°C, and it was this which prevented the experiments being carried out by our chosen method of obtaining R'-donors, which involves exposure to this temperature. In order to obtain a thermoresistant clone from strain 569B, the latter was used as recipient in a cross with strain VT5103. The thermosensitivity of strain VT5103 is due to the presence of phage Mu cts 62 in the his-region of the chromosome. Crossing the two thermosensitive strains VT5103 × 569B, with selection of thermoresistant recombinants made it possible to construct the thermoresistant strain 569BTR.

Next, plasmid RP4::Mu cts 62 was transmitted to strain 569BTR in a conjugation cross VT5103 × 569BTR. The transconjugants were treated with acridine orange (10 μg/ml, with an exposure of 18 h) to "cure" them from the plasmid. Thermosensitive clones which had lost the

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TABLE 1. Bacterial Strains Used in the Work

Strain	Genotype	Presence of plasmid	Source from which obtained
V. cholerae 569B	Prototroph, str	—	From Stavropol' Antiplague Institute
VT 5101	Prototroph, str	RP4: :Mu cts 62	Obtained during this research
VT 5103	ilv arg str ts	RP: :Mu cts62	Cross VT5103 x RB31 as a his^+ ts recombinant
VT 5104	Prototroph, str lysogenic for phage Mu cts 62	RP4: :Mu cts62	Cross VT5103 x 569BTR
RV 31	ilv his arg tox str	—	Parker (USA)
VT 5105	ilv his arg tox str rif	—	Selection of spontaneous Rif^r mutants of strain RV31
VT 5106	ilv arg str tox rif	RP4: :his+	Obtained during this research
VT 5107	ilv arg str tox rif	RP4:his+	Obtained during this research
V. el tor 1621	met lys his		Parker (USA)

TABLE 2. Mobilization of Chromosomal Marker his in VT5104 × VT5105 Cross under Different Conditions of Induction of Prophage Mu cts 62

Expt. No.	Experimental conditions	Selective markers	Number of recombinants in 1 ml of washings from filter	Frequency of transmission of his^+ allele to donor's cell	Frequency of mobilization to transmitted plasmid
1	Crossing at 37°C for 24 h (semi-induction prophage)	Km	9600		
		his	500	$5 \cdot 10^{-7}$	$5 \cdot 10^{-2}$
		his Km	400	$4 \cdot 10^{-7}$	$4 \cdot 10^{-2}$
2	Induction of prophage in donor before crossing at 42°C for 1 h	his	100	$1 \cdot 10^{-7}$	—
		his Km	66	$0{,}6 \cdot 10^{-7}$	—
3	Induction of prophage in donor before crossing at 42°C for 1.5 h	his	98	$1 \cdot 10^{-7}$	—
		his Km	24	$0{,}2 \cdot 10^{-7}$	—

Legend. *) Number of donor's cells in conjugation mixture was 10^9-$4 \cdot 10^9$/ml.

plasmid markers of resistance were selected. Thermosensitivity in the absence of plasmid markers was regarded as the result of lysogenization with the ts-mutant of phage Mu (Mu cts 62).

Plasmid RP4::Mu cts 62 was retransmitted into one of the antibiotic-sensitive ST-clones thus obtained [VT5103 × 569BTR (Mu cts) cross]. As a result, strain VT5104, carrying phage Mu in the composition of the chromosome and plasmid was constructed.

Analysis of conjugation transmission of chromosomal material from strain VT5104 was carried out by crossing this strain with VT5105. The his marker was chosen as the chromosomal marker analyzed for transmission.

During conjugation crosses the experimental conditions were varied by conducting them in two different orders [4]. The first led to semi-induction of phage Mu cts 62 in the course of crossing, the second to induction preceding crossing.

During semi-induction Millipore filters with the donor's and recipient's cells deposited on them were incubated for 24 h on the surface of complete nutrient agar at 37°C. Preliminary induction was carried out by incubating the donor's cells at 42°C for 1 or 1.5 h. After temperature treatment the donor's and recipient's cells were incubated on the surface of complete nutrient agar at 32°C for 24 h. In both orders of the experiment the plasmid was retransmitted with a frequency of 10^{-6}-$5 \cdot 10^{-6}$, calculated per donor's cell. As Table 2 shows, the best results were obtained by crosses done at 37°C.

Analysis of the His^+-transconjugants selected in experiments with crossing at 37°C showed that 11.5% of them (12 of 105 studied) carry resistance markers, and seven of them were thermosensitive, i.e., carried plasmid RP4:Mu cts 62.

To test whether the chromosomal his gene is incorporated into the plasmid, two thermoresistant transconjugants, possessing histidine-independence and plasmid resistance markers (strains VT5106 and VT5107) were used as donors in crosses with *Vibrio el tor* 1621 (Table 3).

It will be clear from Table 3 that the number of recombinants formed during selection on various selective media did not differ significantly. Tests of purified clones grown on medium without histidine showed that they all carry plasmid resistance markers (Km, Ap, Tc). Clones

TABLE 3. Transmission of his^+ Allele and Plasmid Resistance Marker Km during Cross between Strains of *V. cholerae* and *V. el tor*

Donor	Recipient	Selective markers	Number of recombinants in 1 ml of washings of conjugation mixture from filter at undermentioned dilutions		
			undiluted mixture	1:10	1:100
VT5106	1621	his	800	104	6
		his Km	1 000	130	12
		Km	880	136	18
VT5107	1621	his	> 10 000	2000	160
		his Km	> 10 000	880	112
		Km	> 10 000	760	170

Legend. Results of one typical experiment are given.

selected with respect to the kanamycin marker, as well as those to histidine and kanamycin markers, also carried other resistance markers of plasmid RP4, i.e., they were Km Ap Tc His^+.

Combined 100% transmission of plasmid markers and of the chromosome fragment carrying the his^+ gene, which took place independently of the method of selection of the transconjugants, suggests that strains VT5106 and VT5107 are donors of R'-factors. Effective transmission of R'-factors by these strains was observed in experiments conducted by a procedure which followed that of transmission of plasmid RP4::Mu cts 62. During keeping of the strains, however, their ability to serve as donors of R'-factors is lost, evidently due to the recombination processes which function in cholera vibrios (as yet no rec A strains of *V. cholerae* have been obtained [5]). Meanwhile strain VT5104 preserves phage Mu ts 62 in a stable form, in the composition both of the chromosome and of plasmid RP4. Thus this or a strain analogous to it can be used as generator of R'-factors.

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