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A lethal factor in a strain of *Vibrio El Tor**

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The advantage in using membrane filters for crosses between P^+ (donor) and P^- (recipient) strains of *Vibrio cholerae* (Bhaskaran, 1964; Bhaskaran, Sinha & Iyer, 1967) is probably due to more frequent and effective contacts between the cells on the surface of the membrane than is possible in fluid media with actively motile cells of this species. The transfer of the fertility (P) factor itself, as well as the yield of genetic recombinants, was considerably improved by this procedure.

It seemed worthwhile, therefore, to utilize this technique for crosses between strains of *V. cholerae* and *V. El Tor* which have given irregular and often negative results in the past. In particular, P^+ strains of *V. El Tor* were never shown to yield recombinants with P^- strains, either of *V. cholerae* or *V. El Tor*, when tested by conventional procedures such as plating mixtures of washed suspensions of parental strains on selective minimal media.

As a preliminary to this, mixtures of different P^+ and P^- strains (in pairs, containing *c.* $2{\cdot}5 \times 10^9$ cells of each strain derived from 4 h nutrient broth cultures) were fixed by filtration on membrane filters ('Metricel'; pore size, 0·45 μ; diam., 47 mm; Gelman Instrument Co., Ann Arbor, Michigan, U.S.A.) and the proportion of P^- cells acquiring the P factor was determined, at intervals of time, when these membranes were incubated at 37 °C on the surface of sterile, moist nutrient agar plates. This was done by resuspending the culture (on the surface of the membrane) in fluid minimal medium and plating its dilutions on nutrient agar plates for the isolation of discrete colonies of the original P^- strain. When this strain was streptomycin resistant, as it was in most cases, nutrient agar containing streptomycin (500 μg/ml) was used. Twenty randomly selected colonies, which were the progeny of the original P^- parent, were tested for the presence of the P factor which may have been acquired by conjugation with the P^+ partner on the membrane. The test consisted in placing a loopful of these cultures (in nutrient broth) on a semi-solid nutrient agar seeded with an indicator (P^-) strain of *V. cholerae* (162/p.) After overnight incubation at 37 °C, P^+ cultures were identified by a hazy clearing around the periphery of their growth (Bhaskaran, 1964). In our experience, this activity (possibly due to the production of a bacteriocine) is invariably associated with donor ability in *V. cholerae* (but not in *V. El Tor*).

The results are recorded in Table 1. It is clear that the transfer of P factor was less efficient when one or the other partner was *V. El Tor*, whereas, between

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V. cholerae strains the transfer was quickly achieved. The advantage of using membrane filters in such studies is obvious when compared to results obtained in nutrient broth controls containing identical cell suspensions in 1 ml. of medium.

When these studies were in progress, a curious phenomenon was observed in which crosses involving a P+ strain *V. El Tor* (T 10/119 P+) appeared to be lethal to its partner. This could not be attributed to the P factor as the original P− strain (T 10/119) was also lethal. The lethal effect was detected and estimated quantitatively by the reduction in viable count of the sensitive (to the lethal

Table 1. *Transfer (given in percentages) of P factor from P+ to P− strains of* Vibrio cholerae *by conjugation on membrane filter*

Transfer of P factor		Period of contact on membrane filter (min)					Nutrient broth (control) 60 min contact in 1 ml of medium
From P+ strain	To P− strain	0	10	20	30	60	
V. cholerae V 58 str-r	*V. cholerae* V 63 str-s	0	45	80	90	95	10
V. cholerae V 63 str-s	*V. cholerae* V 58 str-r	0	35	60	90	100	5
V. cholerae V 63 str-s	*V. El Tor* T 27 str-r	n.d.	n.d.	n.d.	5	10	5
V. El Tor T 27 str-s	*V. cholerae* V 63 str-r	n.d.	n.d.	n.d.	15	70	5
V. El Tor T 27 str-s	*V. El Tor* T 50/321 str-r	n.d.	n.d.	n.d.	30	50	10
V. El Tor T 50/321 str-s	*V. El Tor* T 27 str-r	n.d.	n.d.	n.d.	5	10	5

n.d.: not done; str-s: streptomycin sensitive; str-r: streptomycin resistant (500 μg/ml). Percentages based on number of P+ strains in a random test of twenty colonies.

Note. Vibrio El Tor comprise a species generally like *V. cholerae* in morphological, biochemical and antigenic characters, but is distinguished by its ability to produce a filterable haemolysin, resistance to a lytic phage (group IV, Mukerjee) and a few other laboratory criteria. Since 1961, *V. El Tor* has been the predominant organism responsible for cholera outbreaks in various areas of South-East Asia.

effect) strain after 30 min. contact on a membrane at 37° C. In these experiments, two membranes were embedded with identical mixtures, containing cell suspensions of T 10/119 and the test strain, derived from 4 h broth cultures. One membrane was immediately resuspended in fluid minimal medium and the other after 30 min. incubation at 37 °C. Viable counts of each were performed, with respect to the test strain, and the reduction observed was calculated. To facilitate this, only streptomycin resistant mutants of test strains were employed, in contrast to T 10/119 which was streptomycin sensitive, so that viable counts could be performed directly on nutrient agar containing streptomycin (500 μg/ml). The presence of streptomycin in the medium used for colony counts did not introduce

any fallacy since similar counts were obtained when minimal agar medium (without streptomycin) was used. For instance, in the cross T10/119 (*str-s his ilv*) × InV505SR (*str-r met thr*), the counts obtained on minimal agar with methionine and threonine were similar to those obtained on nutrient agar with streptomycin.

It will be seen (Table 2) that *V. El Tor*, strain T10/119 proved lethal by this technique to two strains of *V. cholerae*, a non-cholera vibrio strain and two strains of *Escherichia coli*. The reduction in viable counts varied from 99·8 to

Table 2. *Lethal effect of* Vibrio El Tor, *strain T10/119 on test strains*

	Cross		Viable count of strain B Contact on membrane for 30 min		
	Strain A	Strain B			
No.	(*c.* 5×10^9 cells)	(*c.* 5×10^8 cells)	Expt. no.	Initial	Final
1	*V. El Tor* T10/119	*V. cholerae* In V505 SR	1	$5{\cdot}6 \times 10^8$	$7{\cdot}2 \times 10^6$ (1·3)
			2	$1{\cdot}2 \times 10^9$	$2{\cdot}4 \times 10^6$ (0·2)
			3	$7{\cdot}7 \times 10^8$	$3{\cdot}0 \times 10^6$ (0·4)
			4	$6{\cdot}7 \times 10^8$	$5{\cdot}2 \times 10^6$ (0·8)
2	*V. El Tor* T10/119	*V. cholerae* 5/60 SR (T10/119)	1	$4{\cdot}6 \times 10^8$	$1{\cdot}3 \times 10^7$ (2·8)
			2	$6{\cdot}5 \times 10^8$	$1{\cdot}9 \times 10^7$ (2·9)
			3	$6{\cdot}0 \times 10^8$	$2{\cdot}3 \times 10^6$ (0·4)
3	*V. El Tor* T10/119	Non-cholera-vibrio NCV 152 SR	1	$6{\cdot}5 \times 10^8$	$4{\cdot}4 \times 10^6$ (0·7)
			2	$4{\cdot}9 \times 10^8$	$2{\cdot}1 \times 10^7$ (4·3)
4	*V. El Tor* T10/119	*E. coli* 58-161 SR F⁻	1	$7{\cdot}9 \times 10^8$	$1{\cdot}9 \times 10^7$ (2·4)
			2	$2{\cdot}5 \times 10^8$	$1{\cdot}3 \times 10^7$ (5·2)
5	*V. El Tor* T10/119	*E. coli* W677 SR F⁻	1	$4{\cdot}3 \times 10^8$	$2{\cdot}0 \times 10^7$ (4·7)
			2	$1{\cdot}2 \times 10^8$	$1{\cdot}9 \times 10^7$ (15·8)
6	*V. El Tor* HK T10/119	*V. cholerae* In V505 SR	1	$1{\cdot}2 \times 10^9$	$1{\cdot}2 \times 10^9$
7	*V. El Tor* HK T10/119	*V. cholerae* 5/60 SR	1	$2{\cdot}1 \times 10^9$	$2{\cdot}8 \times 10^9$
8	*V. El Tor* HK T10/119	*E. coli* 58-161 SR F⁻	1	$3{\cdot}1 \times 10^8$	$4{\cdot}1 \times 10^8$

HK = heat-killed at 56° C for 30 min. ( ) = percentage of viable cells.

95·7 % with vibrio strains and from 97·6 to 84·2 % in *E. coli*. The lethal effect was however, never complete, although the survivors proved to be sensitive to T10/119 in subsequent tests.

Since strain T10/119 was observed to be lysogenic, a strain of *V. cholerae* (5/60SR) lysogenized with the phage isolated from T10/119, 5/60SR(T10/119), was also included in the tests to rule out the possibility of zygotic induction resulting from the transfer of prophage from T10/119 to strains sensitive to this phage with a consequent fall in viable count. It will be seen in Table 2 that this strain was as sensitive to the lethal effect as the other test strains which were resistant to this phage. Strain T10/119 did not appear to carry any other prophage.

The other possibility may be that an autonomous bacteriocinogenic factor was transferred from T10/119 to sensitive strains leading to lethal synthesis in the

recipient. This has not been excluded, although it seems certain that strain T10/119 does not liberate any bacteriocine to a demonstrable extent during growth in fluid or on solid media. All experiments, based on methods described for colicine testing, proved futile in demonstrating the lethal effect in the absence of live cells. Since vibrio strains are proteolytic, it is not unlikely that any bacteriocine produced by them may be inactivated rapidly.

The probability that the lethal effect was a sequel to conjugation was further supported by the finding that cells of T10/119 killed at 56 °C for 30 min and crossed with sensitive strains on membrane did not prove lethal to the latter (Table 2). This did not occur also in other experiments with live cultures of T10/119 done at 4 °C instead of 37 °C. It may be mentioned that under similar circumstances transfer of P factor does not take place between *V. cholerae* strains.

Three other strains of *V. El Tor* were tested to find out if they were sensitive to the lethal effect of T10/119, or alternatively, whether they themselves displayed any lethal activity. Of these, only one (F17/50) proved resistant while the rest were sensitive to T10/119. None of these strains (including F17/50) displayed any lethal activity on the test strains. As may be expected, mutants of T10/119 (T10/119SR and T27SR) were unaffected by crosses with their parent.

These observations are suggestive that a lethal factor, probably transmissible by conjugation, may be present in *V. El Tor*, strain T10/119, which is reminiscent of the 'mate killer' trait in *Paramecium aurelia* (Siegel, 1953). Further work is necessary to determine whether the lethal factor is a plasmid determining the synthesis of a bacteriocine or a different entity.

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