

CONJUGATION OF *VIBRIO CHOLERAE* STRAINS ON MEMBRANE FILTERS

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AS in *Escherichia coli*¹ and *Salmonella typhimurium*,² conjugation in *Vibrio cholerae* is mediated by a fertility factor, designated as the P-factor.³ This factor is itself transmitted from strain to strain by conjugation. Genetic recombinants can be isolated from crosses between P⁺ and P⁻ strains.^{4,5} It appeared that P⁺ strains functioned as gene donors while P⁻ strains served as recipients.^{5,6}

Because of their active motility, conjugation between *V. cholerae* cells is liable to frequent interruptions. In the case of F-factor mediated conjugation in *E. coli*⁷ and transfer of R factors in enteric bacteria,⁸ such interruptions were minimised and firmer cell-to-cell contact was achieved by fixing the parent cells on membrane filters, the pores of which restricted the bacteria while letting fluids to pass through. It seemed worthwhile to utilise this technique for studying conjugation in *V. cholerae*, and the results of the study carried out so far are summarised here.

In the experiments 5 ml. amounts of 3 hr. broth cultures of the mating strains were mixed and filtered through membrane filters ('Metricel', Gelman Instrument Co., Ann Arbor, Mich.; pore size 0.45 μ). The membranes were then incubated at 37° C. on the surface of nutrient agar plates, the sterile surface of the membrane being in contact with agar. The cells thus mated were resuspended in fluid minimal medium at intervals of time, and tests were carried out to score the frequency of transfer of P factor from P⁺ to P⁻ cells. Experiments were also performed to determine the number of recombinants that can be isolated by plating these cultures on appropriate selective media. Pooled broth suspensions of the same strains, set aside without filtration through membrane, served as controls.

P⁺ and P⁻ derivatives of *V. cholerae* strains, V58 and V63, isolated earlier, were used. When mixtures of P⁺ and P⁻ cells (2.5×10^9 organisms of each) were incubated on membrane filters, transfer of P factor from V58 P⁺ to V63 P⁻ and from V63 P⁺ to V58 P⁻ occurred rapidly; 75–100% of the P strain acquiring the P factor in 30–60 minutes. In nutrient broth controls, such a transfer was detected only in about 10% of the cells in 60 minutes. When cell suspensions recovered from the membranes after 30 minutes incubation were examined

under microscope, clumping of cells could be seen which was suggestive of efficient pairing. This was not so obvious in the controls.

Recombinants were isolated in greater numbers when the cells were plated on selective minimal media after pre-incubation on membrane filters than in controls (Table I).

TABLE I
Comparative frequency of recombinants pre-incubation on membrane filter and in nutrient broth

Cross		Selective markers		No. of recombinants (per 10 ⁸ cells of the pool)	
Strain V58	Strain V63	Strain V58	Strain V63	Membrane filter (1 hr.)	Nutrient broth (1 hr.)
P ⁺	P ⁻	leu ⁺	ilv ⁺ arg ⁺ his ⁺	63	14
		pur ⁺ leu ⁺	arg ⁺	66	7
P ⁻	P ⁺	leu ⁺	ilv ⁺ arg ⁺ his ⁺	55	1
		pur ⁺ leu ⁺	arg ⁺	18	8
P ⁺	P ⁺	leu ⁺	ilv ⁺ arg ⁺ his ⁺	9	3
		pur ⁺ leu ⁺	arg ⁺	7	1
P ⁻	P ⁻	leu ⁺	ilv ⁺ arg ⁺ his ⁺	1*	nil
(Control)		pur ⁺ leu ⁺	arg ⁺	nil	nil

Markers of V58 = str-s pur⁺ ilv⁻ O-Og arg⁻ leu⁺ his⁻
 ,, V63 = str-r pur⁻ ilv⁺ O-In arg⁺ leu⁻ his⁺
 pur = purine; ilv = valine + isoleucine; arg = arginine; leu = leucine; his = histidine; O-Og = O antigenic type Ogawa; O-In = O antigenic type Inaba; str-s = streptomycin-sensitive; str-r = resistant to streptomycin (500 μ g/ml.) (+) indicates independence. () indicates dependence. * leu mutant of V63.

A study of the unselected markers of the recombinants thus isolated showed little evidence of unidirectional transfer of genetic material, as recombinants seemed to arise from both the strains employed in the cross. Because of the high frequency of transfer of P factor on membrane filter, it was possible that these recombinants resulted both from P⁺ \times P⁻ and P⁺ \times P⁺ matings.

In order to investigate this, experiments were carried out as described in *E. coli*,⁹ employing

a streptomycin-sensitive P^+ and a streptomycin-resistant P^- strain for such crosses and using selective media containing streptomycin (100 μ g./ml.) for the isolation of recombinants. On such media, only the P^- strain can survive and be capable of giving rise to recombinants. Results of these experiments are given in Table II.

TABLE II

Differential effect of streptomycin on fertility in *Vibrio cholerae* crosses

Cross		Selective markers		No. of recombinants (per 2×10^8 cells of the pool)	
Strain V58	Strain V63	Strain V58	Strain V63	Pre-incubation on membrane filter (30 min.)	Control
P^+	P^-	pur^+	$str-r$	102	17
		leu^+	$str-r$	103	4
P^-	P^+	pur^+	$str-r$	6	5
		leu^+	$str-r$	1	1
P^+	P^+	pur^+	$str-r$	6	3
		leu^+	$str-r$	11	3

(See Table I for markers of strains and symbols used)

It will be seen that recombinants were isolated in large numbers only from $P^+ \times P^-$ cross after pre-incubation on membrane filter. If the membrane filter technique was omitted,

as in controls, there was a considerable reduction in their numbers. Such a reduction was also seen in reversed $P^+ \times P^-$ and $P^+ \times P^+$ crosses. These findings provide strong evidence for one-way transfer of genetic material in *V. cholerae*, as is known in *E. coli*⁹ and *S. typhimurium*.¹⁰

It is obvious that the membrane filter technique should permit detailed studies on the kinetics of the mating process in *V. cholerae* and also facilitate crosses between *V. cholerae* and *V. el Tor* strains and between *V. el Tor* strains which presented difficulties in the past.¹¹

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1. Lederberg, J., Cavalli, L. L. and Lederberg, E. M., *Genetics*, 1952, **37**, 720.
2. Stocker, B. A. D., In: Hayes, W. and Clowes, R. C., ed., *Microbial Genetics*. Tenth Symposium of the Society for General Microbiology held at the Royal Institution, London, University Press, Cambridge, April 1960. p. 1.
3. Bhaskaran, K., *Indian J. Med. Res.*, 1959, **47**, 253.
4. —, *J. gen. Microbiol.*, 1960, **23**, 47.
5. —, *Bull. Wld. Hlth. Org.*, 1964, **30**, 845.
6. — and Iyer, S. S., *Nature (Lond.)*, 1961, **189**, 1030.
7. Matney, T. S. and Achenbach, N. E., *J. Bact.*, 1962, **84**, 874.
8. Smith, D. H. and Armour, S. E., *Lancet*, 1966, **2**, 15.
9. Hayes, W., *Nature (Lond.)*, 1952, **169**, 118.
10. Smith, S. M. and Stocker, B. A. D., *Brit. med. Bull.*, 1962, **18**, 46.
11. Iyer, S. S., *Ph.D. Thesis*, Banaras Hindu University, 1966.

A DENSE FRUITED MUTATION IN INDUCED AUTOTETRAPLOID BROWN SARSON (*BRASSICA CAMPESTRIS* var. BROWN SARSON)

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S EED yield in induced autotetraploid brown sarson, *Brassica campestris* var. brown sarson, could be increased not only by selecting plants for larger number of branches, larger number of seeds per silique and higher seed weight but also by selecting plants having a larger number of silique per branch. Such a "dense fruiting" mutation, arising as a result of radiation, is reported in this note.

Induced polyploids have been of economic value in only a few species principally those grown for their vegetative or floral parts. Autotetraploids of some crop plants have proved valuable because of their intrinsically superior qualities conferred by polyploidy alone. The most successful of these is the cereal rye which is competing successfully with diploids by virtue

of its large kernel size, superior sprouting ability and better baking quality of the grain due to its high protein content. (Muntzing).⁵ It has, however, some disadvantages such as reduced tillering, lower seed setting, tall straw which makes harvesting with combines difficult and also the necessity of isolating it from the diploids with which it crosses readily resulting in sterile triploids. Similar advantages and disadvantages like low seed setting are also found in other induced autotetraploid cereals and oil crops. Some of these defects have been partly rectified.

Employing the mass pedigree method of breeding Parthasarathy and Rajan⁶ considerably improved the fertility of tetraploid population of *Brassica campestris* var. toria which was