Genetic Recombination in *Vibrio cholerae*

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SUMMARY: Some pairs of genetically marked strains of *Vibrio cholerae* when grown together and plated under selective conditions give rise to greater number of colonies than either strain plated alone; in the case of other pairs in which this phenomenon did not occur, large number of presumptive recombinants were obtained when one of the strains was made lysogenic with a particular phage. Although the small number of markers made it impossible to do mapping studies, the evidence is strongly suggestive of recombination, probably phage-mediated transduction.

The literature on cholera contains many reports on the diverse variability of *Vibrio cholerae* which appeared to White (1937) to be without parallel in bacteriological lore. He examined some of these reports and made personal studies of some of the alleged variants, pointing out discrepancies which could not be explained. The only changes in this organism which were fully confirmed in later years were antigenic variations from Ogawa to Inaba and from smooth to rough forms (Shrivastava & White, 1947), which appeared to result from mutations (Bhaskaran & Gorrill, 1957). It seemed that genetic studies based on the techniques recently evolved for *Escherichia coli* and *Salmonella* were necessary to show how far the specific characters of *V. cholerae* may be altered. In this work genetical recombination is reported in *V. cholerae* and a possible mechanism suggested.

MATERIALS AND METHODS

Strains of Vibrio cholerae. A rough strain 129, requiring purine (Pu⁻) for growth, was found to be lysogenic for a phage, also designated 129. For the sake of consistency with the terminology used in other strains this strain was designated 129 (129)⁺ to indicate the presence of phage 129. The following strains were found to be sensitive to phage 129: 6 (Inaba type, prototrophic), 20 (Inaba type, prototrophic), C₄ (Inaba type, prototrophic, non-motile), S^R (streptomycin-resistant), 23 (Ogawa type, prototrophic) 121 (Ogawa type, methionine-requiring (M⁻)) and 121 S^R, a streptomycin-resistant derivative of 121. These strains could be made lysogenic with phage 129 and, for example, strain 20 after lysogenization would be designated 20 (129)⁺.

The antigenic type of these strains and the presumptive recombinants isolated in the experiments was determined by slide agglutination tests with suitably diluted mono-specific 'O' antisera (Ogawa, Inaba and Rough).

Media. *Nutrient broth* was used for the cultivation of *Vibrio cholerae*. It contained peptone (British Drug Houses Ltd., England), 0.5 % (w/v); Lab-Lemco (Oxo Laboratories, England), 0.1 % (w/v); sodium chloride (A.R.) 0.5 % (w/v); yeast extract (Difco Laboratories, Detroit), 0.1 % (w/v) in distilled

water, adjusted to pH 8 and sterilized in the autoclave at 15 lb./sq.in. for 15 min.

Nutrient agar. This was prepared by adding agar powder (British Drug Houses Ltd., England), 1.5% (w/v) to the nutrient broth.

Minimal agar. This was the minimal agar of Bhaskaran & Rowley (1956), containing ammonium sulphate and glucose as sole nitrogen, carbon and energy sources.

Technical procedures for recombination

Recombination for prototrophs. Ten ml. amounts of broth, distributed in 50 ml. conical flasks to provide a shallow layer of the medium, were seeded with 10^9 organisms of each strain individually or together. After 15 hr. incubation at 37° , the cultures were centrifuged at 2500 rev./min. for 15 min. and the deposits were washed with minimal medium. The final deposits were suspended in 1 ml. fluid minimal medium from which 0.1 ml. were seeded on minimal agar. Viable counts were made at the same time. After 72 hr. incubation at 37° , the numbers of prototrophic colonies which appeared from each culture and from the mixture were counted.

Recombination for streptomycin-resistant motile flares. Transduction of motility was studied in detail by Stocker, Zinder & Lederberg (1953). The method adopted for recombination in the present experiments was essentially the same as that for obtaining prototrophs but loopfuls of the centrifuged deposits were streaked on semi-solid gelatin agar (Edwards & Bruner, 1942) to which streptomycin had been added to a concentration of $500 \mu\text{g./ml.}$; after 24 hr. incubation at 37° the flares which arose from streptomycin resistant recombinants were counted.

RESULTS

Studies on lysogenic strains of *Vibrio cholerae* showed that strain 121 and its streptomycin-resistant mutant were reliable indicators for several lysogenic strains. When broth culture supernatants of the lysogenic cultures were spotted on a lawn of 0.4% (w/v) agar seeded with the indicator, according to the method of Adams (1950), turbid plaques with narrow clear lytic edges appeared after overnight incubation at 37° . It seemed possible to utilize these indicator strains to identify potential transducing phages among the lysogenic strains by direct crosses with them in the same manner in which genetic transduction was originally discovered in *Salmonella* (Zinder & Lederberg, 1952). With nutritional and other markers differentiating the lysogenic and indicator strains, selective methods would reveal if any genetic exchange occurred between the two.

One out of 26 such crosses proved to be of interest. In this the strains 129 (129)⁺ (purine exacting) and 121 S^R (methionine exacting) were crossed and it will be seen from Table 1 that the number of prototrophs isolated from the mixed culture was higher than in control single cultures and that they appear with frequency of 0.6 in 10^6 . They were lysogenic for phage 129, producing plaques on the parent strain 121 S^R.

These results suggest transfer of methionine independence from 129 (129)⁺ to 121 S^B, possibly mediated by phage 129. Strain 129 (129)⁺ was further crossed with the non-motile strain C₄ by the method described above and gave motile streptomycin-resistant flares and the fact that all the unselected markers were from one parent (Table 2) again suggested the transfer of one marker

Table 1. *Numbers of presumptive recombinants obtained in crosses between strains of Vibrio cholerae, using 10⁹ parent organisms*

Parent strains, and number of colonies produced by each, and by mixture, on minimal medium + streptomycin*				
Lysogenic (presumptive donor†) strains	No.	Non-lysogenic strain	No.	Mixed strains (cross), no.
129 (129) ⁺ (Pu-S ^B)	36†	121 S ^B (M-S ^B)	12†	600†
6 (prototroph S ^B)	1	121 S ^B (M-S ^B)	.	1
20 (prototroph S ^B)	0	121 S ^B (M-S ^B)	.	0
23 (prototroph S ^B)	0	121 S ^B (M-S ^B)	.	0
6 (129) ⁺ (prototroph S ^B)	0	121 S ^B (M-S ^B)	.	95
20 (129) ⁺ (prototroph S ^B)	0	121 S ^B (M-S ^B)	.	234
23 (129) ⁺ (prototroph S ^B)	0	121 S ^B (M-S ^B)	.	102

Parent strains, and number of 'flares' produced by each, and by mixture, in semi-solid agar + streptomycin*

Lysogenic (presumptive donor†) strain	No.	Non-lysogenic strain	No.	Mixed strains (cross no.)
129 (129) ⁺ (motile Pu-S ^B)	0	C ₄ (prototroph non-motile S ^B)	0	++
6 (prototroph motile S ^B)	0	C ₄ (prototroph non-motile S ^B)	0	0
23 (prototroph motile S ^B)	0	C ₄ (prototroph non-motile S ^B)	.	0
6 (129) ⁺ (prototroph motile S ^B)	0	C ₄ (prototroph non-motile S ^B)	.	++
23 (129) ⁺ (prototroph motile S ^B)	0	C ₄ (prototroph non-motile S ^B)	.	++

* 500 µg. ml. † On hypothesis of transduction. ++ indicates numerous confluent flares.

‡ No of colonies on minimal medium without streptomycin.

Table 2. *Details of unselected markers of isolated recombinants obtained in crosses between strains of Vibrio cholerae*

Lysogenic parent strain		Non-lysogenic parent strain		Medium used to select recombinants	Selected markers	No. examined and details of unselected markers of commonest class of recombinant	Other classes
Strain	Characters	Strain	Characters				
129 (129) ⁺	(Rough M+S ^B Pu ⁻)	121 S ^B	(Ogawa M-S ^B Pu ⁺)	Minimal medium	Pu ⁺ , M ⁺	200 Ogawa S ^B	None
6 (129) ⁺	Inaba S ^B			Minimal medium + streptomycin	M ⁺ , S ^B	{ 94 Ogawa	1 Inaba
20 (129) ⁺	(Inaba S ^B)			Semisolid agar + streptomycin	Motility, S ^B	{ 100 Ogawa	None
129 (129) ⁺	(Rough M+S ^B Pu ⁻)	C ₄	(Inaba non-motile S ^B)			{ 20 Inaba proto-	None
23 (129) ⁺	(Ogawa S ^B)					20 Inaba	None

(motility) from 129 (129)⁺ to C₄. It may be remarked that C₄ is a stable non-motile strain and has not so far revealed the occurrence of motile mutants in several tests on semi-solid gelatin agar.

It was not possible to test the role of phage 129 as a transducing agent by means of cell-free lysates since a sufficiently high titre of phage could not be obtained. However, after lysogenizing one of the parent strains it has been

possible to demonstrate crossing in systems previously infertile. Such studies were carried out with strains 6, 20 and 23, none of which could be crossed with 121 S^R to yield streptomycin-resistant prototrophs or with C₄ to yield motile streptomycin-resistant flares; however, derivatives of strains 6, 20 and 23 made lysogenic with phage 129 gave positive results when crossed with 121 S^R or C₄ (Table 1). Here again the unselected markers of the recombinants isolated were those of the non-lysogenic strain (but in the case of the crosses 23 (129)⁺ × 121 S^R and 6 (129)⁺ × C₄ no unselected markers were available for examination). It will be seen from Table 2 that in the cross 6 (129)⁺ × 121 S^R, 94 out of 95 recombinant colonies tested had the unselected antigenic marker of the non-lysogenic strain, whereas in 20 (129)⁺ × 121 S^R, all the colonies tested were like the non-lysogenic strain in this respect. The lone exception was perhaps a streptomycin-resistant mutant of 6 (129)⁺. Isolates from recombination tests for motility gave similar results. It will be seen from Table 1 that the occurrence of streptomycin-resistant mutants in strains 6, 20 and 23 with an inoculum of 10⁹ organisms was quite rare which permitted the use of streptomycin containing media to isolate presumptive recombinants from crosses involving these strains.

DISCUSSION

The existence of genetic mechanisms in bacteria is now well established, although the number of species in which genetic interchanges have been shown to occur is still very limited and the importance of these phenomena in nature has still to be elucidated. The process of genetic exchange between bacterial strains may take place by one of several distinct mechanisms, e.g. transformation (Avery, McCleod & McCarty, 1944), conjugation (Tatum & Lederberg, 1947) and transduction (Zinder & Lederberg, 1952).

The experimental data presented and summarized in Table 1 implicate phage 129 in recombination in *Vibrio cholerae*, since the introduction of phage 129 as a lysogenic phage into a strain can render it fertile. By analogy with *Salmonella* (Zinder & Lederberg, 1952) it can be suggested that recombination takes place by transduction and that phage 129 is the transducing agent. This hypothesis is supported by the lysogenic state of the recombinants, at least in the cross 129 (129)⁺ × 121 S^R, and by the fact that the unselected markers are those of the non-lysogenic strain (Table 2).

However, the evidence, though very strong, is circumstantial and until cell- and DNA-free lysates of phage 129 can be used to transduce characters into sensitive cells the hypothesis of phage-mediated transduction cannot be considered proven.

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