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Biotype-Specific Restriction and Modification of DNA in *Vibrio cholerae*

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By using *Vibrio cholerae* typing phages it was possible to demonstrate that within *V. cholerae* of the O-1 serotype there are at least two biotype-specific DNA restriction and modification systems.

As with many bacteria, bacteriophages have been used in *Vibrio cholerae* as a means of differentiating subtypes. In the case of *V. cholerae* belonging to the O-1 serotype, the subdivision is based primarily on two biotypes: the classical biotype, which has been responsible for the disease for many centuries, and the El Tor biotype, which was isolated at the beginning of this century and is currently the prevailing causative agent of the disease. Mukerjee and coworkers (4–7) showed that strains of the classical biotype could be subdivided into five classes on the basis of sensitivity to four phages (classical I through IV). Similarly, a set of five bacteriophages (El Tor I through V) could be shown to divide the El Tor biotype into six groups (3). In fact the classical IV phage was recommended by the World Health Organization to be the basis for differentiating the two biotypes of O-group 1 *V. cholerae* (2). Part of the specificity of these various phages was thought to be the result of masking or absence of their receptors or of immunity, owing to the strains being lysogens. The work we present here is part of a study which we have undertaken to see whether either was in fact the case, as it seemed likely that an overriding difference, that of DNA restriction, might account for some of the differences observed.

Classical phages I and IV and El Tor phage I were propagated on the classical strain 154 and El Tor strain MAK757 which are, respectively, the standard propagating strains for these phages. These phages were plated in a soft agar (0.7%) overlay at high multiplicity, with 0.1 ml of a midlogarithmic phase culture of the strain of the opposite biotype. The efficiency of plating of the phage on the opposite biotype was seriously impaired (Table 1); however, a few plaques were detected. When these plaques were then picked, reisolated on that strain twice, and then propagated on that strain, high titers of phage were obtained. These new phage stocks (labeled [b] in Table 1) could then no longer efficiently propagate on their correct biotype. Thus, the classical phages I and IV, after they were propagated on the El Tor strain, could no longer plaque efficiently on the classical strain. These results were repeated for the El Tor phage I. This cycle could be repeated back and forth, and the phage would only plaque efficiently on strains of the biotype on which they were last propagated. Thus, we observed restriction and modification of the phage DNA in the same way Arber and Dussoix (1) showed for bacteriophage λ in *Escherichia coli* K12 and *E. coli* B.

To determine whether the restriction and modification we were observing was biotype specific we screened 26 additional *V. cholerae* strains for their sensitivity to the bacteriophage propagated on either strain 154 or strain MAK757. Additionally we examined these strains for the properties classically used to biotype *V. cholerae*: resistance to polymyxin

TABLE 1. Efficiency of plating of *V. cholerae* typing phages on the opposite biotype

Phage	Efficiency of plating on strain:[a]	
	154	MAK757
Classical I	1	1.5×10^{-6}
Classical IV	1	6×10^{-7}
El Tor I	1.2×10^{-8}	1
Classical I[b]	2×10^{-6}	1
Classical IV[b]	6×10^{-7}	1
El Tor I[b]	1	10^{-9}

[a] Strain 154 is of the classical biotype; strain MAK757 is of the El Tor biotype. Strains 154 and MAK757, as well as the typing phages, were obtained from B. C. Deb, National Institute of Cholera and Enteric Diseases, Calcutta, India.

[b] Single plaques of phage which were isolated on the opposite biotype and then propagated on that biotype. Single plaques were picked, put through two further single-plaque isolations, and then propagated in liquid medium. The medium used throughout was brain heart infusion (Difco Laboratories), and all of the incubations were carried out at 37°C with aeration.

TABLE 2. Biotyping *V. cholerae* strains

Strain	Hemolysin[a]	Phage sensitivity: Classical I	Classical I[d]	Classical IV	Classical IV[d]	El Tor I	El Tor I[d]	Biotype predicted by phage restriction pattern[b]	Source[c]
Classical									
154	–	+	–	+	–	–	+	C	A
V58	–	+	–	+	–	–	+	C	B
0162	–	+	–	+	–	–	+	C	B
I162	–	+	–	+	–	–	+	C	B
G2102	–	+	–	+	–	–	+	C	B
569B	–	+	–	+	–	–	+	C	C
Kuki	–	+	–	+	–	–	+	C	B
CA411	–	+	–	+	–	–	+	C	D
CA401	–	+	–	+	–	–	+	C	D
NIH41	–	+	–	+	–	–	–	C	B
NM111	–	+	–	+	–	–	+	C	B
El Tor									
MAK757	–	–	+	–	+	+	–	ET	A
R1MD	+	–	–	–	–	+	–	ET	B
T50	–	–	–	–	–	+	–	ET	B
T51	–	–	–	–	–	+	–	ET	B
Ph6	–	–	–	–	–	+	–	ET	B
Ph7	+	–	–	–	–	+	–	ET	B
Ph8	–	–	–	–	–	+	–	ET	B
Ph9	–	–	–	–	–	+	–	ET	B
Ph10	+	–	–	–	–	+	–	ET	B
Afg4	–	–	–	–	–	+	–	ET	B
Afg6	+	–	–	–	–	+	–	ET	B
017	+	–	+	–	–	+	–	ET	E
AV86	+	–	+	–	–	+	–	ET	B
1633	+	–	–	–	–	+	–	ET	F
1621	+	–	+	–	–	+	–	ET	F
905	+	–	+	–	+	+	–	ET	F

[a] Hemolysin production was measured as lysis of sheep erythrocytes in blood agar plates.

[b] C, Classical; ET, El Tor.

[c] A, B. C. Deb, National Institute of Cholera and Enteric Diseases, Calcutta, India; B, K. Baskaran; C, I. Hug, Cholera Research Institute, Dacca, Pakistan; D, C. Parker, Columbia, Mo.; E, W. F. Verwey; F, J. Ogg, Fort Collins, Colo.

[d] The bacteriophage, for example, classical I, was propagated on a strain of the opposite biotype, in this case El Tor. Ten microliters of a suspension of 10^6 PFU per ml was spotted onto a lawn of the test strain, incubated overnight, and scored for sensitivity (+) or resistance (–).

B, ability to agglutinate chicken erythrocytes, and the production of hemolysin against sheep erythrocytes. All of the classical strains were fully sensitive to polymyxin B (10 and 50 μg/ml) and did not agglutinate chicken erythrocytes (measured by mixing 0.1 ml of log-phase bacteria at 4×10^8 cells per ml with 0.1 ml of 5% washed chicken erythrocytes in saline). The opposite was true for all of the El Tor strains. The other results are summarized in Table 2 along with the biotype which was predicted by using the restriction pattern as a guide for biotyping.

Although the first El Tor isolates were distinctive in possessing a hemolysin, this has been variable in more recent isolates. However, resistance to polymyxin B and hemagglutinin production enable the strains to be readily biotyped. These tests gave identical results to those predicted on the basis of phage restriction. It can also be seen (Table 2) that in the case of some strains one or more of the phages were unable to form plaques, irrespective of the biotype on which they were propagated. These would be candidates for strains lacking the receptors for these phages, although the possibility of phage immunity due to lysogeny still remains. The results also show that classical phage IV, when propagated on strain MAK757, can infect at least some El Tor strains, so that if this phage is to continue to be routinely used for biotyping, care should be taken with its propagation. However, properly maintained, it is still useful for separating strains into classical and El Tor biotypes.

In addition we examined classical phages II

and III and El Tor phages II to V. Not all of the bacteriophages tested responded in the way demonstrated above. Some phages, such as classical phage II, appeared to form host range mutants at low frequency (approximately 10^{-6}) which could plaque equally well on both El Tor and classical strains. This suggests that the phage is not subject to restriction, possibly due to its having a separate modification system capable of overriding the host restriction and modification systems or of being able to block the host system. Other phages, such as El Tor phages II, IV, and V, could not form plaques at all (efficiency of plating less than 10^{-8}) on classical strains. In the case of phage II this is because the receptors do not exist on these strains. An additional phage, the lysogenic phage CP-TI (8), was also tested on the strains, and it plaqued with equal efficiency on nearly all of the strains, with the exception of strain 1633, which was already lysogenic for this phage.

Our results demonstrate the existence of two biotype-specific DNA restriction and modification systems in *V. cholerae* strains of the O-1 serotype. To generalize that the restriction and modification are biotype specific may be questioned, because only two strains have been used for making lysates. However, it must be pointed out that these two strains are the basis for phage biotyping in *V. cholerae*.

The fact that there are strains which are resistant to phage no matter which biotype is used for propagation demonstrates that there is considerable variability in *V. cholerae* serotype O-1 strains. We are presently trying to determine the receptor specificity of these phages in the hope of being able to understand at least part of this variability.

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LITERATURE CITED

1. **Arber, W., and D. Dussoix.** 1962. Host specificity of DNA produced by *Escherichia coli*. I. Host controlled modification of bacteriophage λ. J. Mol. Biol. **5**:18–36.
2. **Barua, D., W. Burrows, and J. Gallut.** 1970. Principles and practice of cholera control, p. 128–129. *In* Public Health Paper no. 40. World Health Organization, Geneva.
3. **Basu, S., and S. Mukerjee.** 1968. Bacteriophage typing of *Vibrio eltor*. Experientia **24**:299–300.
4. **Mukerjee, S., D. K. Guha, and U. K. Guha Roy.** 1957. Studies on typing of cholera by bacteriophage. Part I. Phage typing of *Vibrio cholerae* from Calcutta epidemics. Ann. Biochem. Exp. Med. **17**:161–176.
5. **Mukerjee, S., U. K. Guha Roy, D. K. Guha, and B. C. Rudra.** 1959. Studies on typing of *Vibrio cholerae* by bacteriophage. Part II. Further observations with strains from Calcutta epidemics. Ann. Biochem. Exp. Med. **19**:115–124.
6. **Mukerjee, S., U. K. Guha Roy, and B. C. Rudra.** 1960. Studies on typing of cholera vibrios by bacteriophage. Part III. Phage-typing of strains of Calcutta epidemics of 1959. Ann. Biochem. Exp. Med. **20**:181–188.
7. **Mukerjee, S., U. K. Guha Roy, and B. C. Rudra.** 1963. Studies on typing of cholera vibrios by bacteriophage. Part IV. Variations in the phage type of *Vibrio cholerae* in the Calcutta epidemics of 1960–1962. Ann. Biochem. Exp. Med. **23**:201–208.
8. **Ogg, J. E., T. L. Timme, and M. M. Abmohammad.** 1981. General transduction in *Vibrio cholerae*. Infect. Immun. **31**:737–741.