

## **5. Studies on *Vibrio cholerae* phages**

### **5.1 Nationwide screening of phage types of *V. cholerae* O1 biotype EITor**

Investigator: B. L. Sarkar.

During the period under study, a total of 413 strains of *V.cholerae* were received from different parts of the country including West Bengal for serotyping, biotyping and phage typing. Of these, 306 (74.09%) representative strains confirmed as *V. cholerae* O1 biotype EITor were included in phage typing study. This year, highest number of strains was received from Maharashtra state. Majority of the strains belonged to Inaba (63%). For the last couple of years, Ogawa was the dominant serogroup. A total of 30 (9.8%) strains were found to be untypeable with the conventional scheme of Basu and Mukerjee. These strains were grouped under type 2 with Basu and Mukerjee scheme. Using the new scheme, all of these strains were found to be typeable and could be clustered into a number of distinct types of which majority were grouped under type 27 (91.83%) followed by type 23 (2.61%), type 19 (1.30%), type 26 (0.98%) respectively. It has been observed that type 27 was the predominant phage type circulating in this country.

### **5.2 Further application of phage typing scheme for discriminating strains of *Vibrio cholerae* O139**

Investigator: B. L. Sarkar

During the current year, not a single strain was isolated from any parts of the country except Delhi. A total of 8 strains of *V. cholerae* O139 were received for phage typing study from Delhi. All these strains were grouped under type 1.

### **5.3 Molecular analysis of *Vibrio cholerae* bacteriophages: cloning and sequencing of phage DNA**

Investigator: B.L. Sarkar

N-4  $\Phi$ , a lytic bacteriophage of EITor O1 typing scheme was selected for complete nucleotide sequencing. Genomic DNA was subjected to enzymatic digestion with a twenty eight restriction enzymes. The size of the DNA was 40 kb as estimated with *Hind*III and *Eco*RV. The phage DNA was randomly sheared using an ultrasonic disintegrator and was controlled in such a way to generate fragments ranging from 0.2-1kb with most between 500-600bp regions. Sheared DNA fragments were treated with Mungbean nuclease to generate blunt ends and electrophoresed onto agarose gels. DNA fragments ranging between 500 and 600bp sizes were cut out from the gel and recovered from the agarose blocks. Gel-eluted fragments ranging between 500 and 600 bp was ligated to *Eco*RV digested cloning vector pZero-2.1. Transformants were selected on kanamycin (50 $\mu$ g/ml) plates containing 1 mM IPTG. A total of 470 transformants were arbitrarily picked up for further study. Purified plasmid DNA was isolated from randomly selected 170 clones and tested for the presence of insert. Results showed that among these 170 clones, contained inserts with sizes ranging between 500 and 600bp. Here, universal M13 primers were used as sequencing primer. So far, 130 clones were sequenced and nucleotide sequence data were assembled using the software DNASTAR and SEQUENCHER. Assembly of the reads resulted into many contigs with maximum and

minimum sizes are 9000 and 500 bp respectively. Numerical addition of the sizes of all the contigs could cover 36 kb region of the phage. Despite the fact that existence of many gaps to join these contigs, BLAST searches were carried out with individual contigs. Interestingly, nucleotide sequence data generated from most these contigs did not show any significant match to the data available in the public domain databases. Therefore, speculation ran high whether or not the genome of these phages may be unique in nature. The studies are underway to complete the whole genome sequence of said phage.

#### **5.4 Studies on the effect of *Vibrio cholera* phages on *V. cholerae* in RITARD model**

Investigator: B. L. Sarkar

During the period under study, *Vibrio cholerae* O1 strain MAK 757 and the cocktail phage of *V. cholerae*O1 (five phages) were challenged in Rabbit Ileal Loop (RIL) model. Two sets of rabbits were used in this purpose, one was infected with only *V. cholerae* MAK 757 strain and in another one MAK 757 and a cocktail of respective vibriophages were used. In both the rabbits, diarrhoeagenic inflammation was observed but it was found to be less in the rabbit where vibriophages were challenged.

After that, similar experiment was carried out in RITARD model. In one set (control), the rabbit was infected with only *V. cholerae* MAK 757 strain. In another set, the rabbit was challenged with MAK 757 with cocktail phage of *V. cholerae* O1. Severe cholera or diarrhea with voluminous stool was observed in the control set. On the other hand, there was no cholera or any histological abnormality in the experimental set. The studies are underway to confirm that cholera phage can be an alternative to antibiotic as phage therapy.

**Nationwide screening of phage types of *V. cholerae* 01 biotype**

**Serotype, Biotype & phage type results of *V. cholerae* 01 biotype EITor strains during  
2006-2007**

STATE	STRAINS	NEW PHAGE TYPING SCHEME											Basu & M		
		4	12	13	15	19	21	22	23	24	25	26	27	T4	UT
Delhi	21												21	21	0
Gujrat	105		1			2			4			2	96	93	12
Karnataka	10												10	10	0
Maharashtra	156	1		1	1	2	1	1	3	1	1	2	142	148	8
Rajasthan	1												1	1	0
Tamil Nadu	10												10	10	0
Uttar Pradesh	2												2	2	0
West Bengal	31			1		1			2				27	21	10
<b>Total</b>	<b>336</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>1</b>	<b>5</b>	<b>1</b>	<b>1</b>	<b>9</b>	<b>1</b>	<b>1</b>	<b>4</b>	<b>309</b>	<b>306</b>	<b>30</b>