

## SHORT ARTICLES

### ISOLATION OF RAPIDLY LACTOSE-FERMENTING *VIBRIO CHOLERAE* STRAINS

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WHEN tested by the usual procedures, *Vibrio cholerae* strains usually appear as non-fermenters of lactose after incubation overnight, but on further incubation some strains produce acid from lactose. During the 1970 cholera season in Calcutta—from April onwards—we observed that many strains fermented lactose promptly.

#### MATERIALS AND METHODS

In a study of diarrhoea in Calcutta, sponsored jointly by the Indian Council for Medical Research, the World Health Organization, and the West Bengal Government, *V. cholerae* strains were isolated either on thiosulphate citrate bile-salt sucrose agar (TCBS Agar, Nissui Seiyaku Co., see Kobayashi *et al.*, 1963) or on Vibrio Agar (Nissui, see Tamura, Shimada and Prescott, 1971). This was followed by slide agglutination with Gardner and Venkatraman's O-1 serum, and Inaba and Ogawa sera, and inoculation into triple-sugar iron agar (TSI Agar, Baltimore Biological Laboratories) and Lysine Decarboxylase Medium (Nissui). After incubation overnight, changes in the butt and slant were observed, and the oxidase test was performed on growth from the TSI slant.

Strains that were to be studied further were maintained in stock culture. Later, transfers were made on to nutrient agar plates, but no more than three passages were done before the following tests were performed.

Carbohydrate fermentations were tested for by inoculating the strains from a 3-hr culture in Trypticase Soy Broth (BBL) into peptone water containing 1 per cent. sucrose, mannose, arabinose or lactose respectively, with Andrade's indicator added. Readings were taken daily for 7 days after incubation at 37°C. Sixteen strains were also tested for fermentation of lactose in concentrations of 2, 3, 4 and 5 per cent. in the above medium and readings were taken after incubation overnight. The Voges-Proskauer (VP) test was done on cultures in a semi-solid medium (Eiken Chemical Co., Ltd, Tokyo) by Barritt's method. Indole production was tested in Sakazaki's (1954) sulphide-indole-motility medium (SIM, Nissui). The haemolysis test was carried out by the methods of Feeley and Pittman (1963) and Sanyal *et al.* (1971) in Heart Infusion Broth (Difco) to which 1 per cent. glycerol was added for the latter method. Chicken red-cell agglutination was tested for by the method of Finkelstein and Mukerjee (1963).

Group-IV classical cholera phage sensitivity and polymyxin-B sensitivity were tested by the methods described by Mukerjee (1963) and Roy *et al.* (1965) respectively.

#### RESULTS

During the months of April and May, 131 vibrio strains were isolated. In 57 of these the slants turned yellow along with the butt in the TSI medium, indicating that lactose was also fermented. All 131 strains tested belonged to Heiberg group I and were agglutinable by

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cholera O-I serum and Inaba specific serum, but not by Ogawa specific serum or by normal saline. In all 57 cases it could be shown that lactose was fermented after overnight incubation in the peptone water sugar medium as well. In a few cases only a little acid was produced, but after 48 hours' incubation all strains gave strongly positive results. No other changes were observed during the following 7 days of incubation. Ten strains tested with higher concentrations of lactose gave identical results.

All 57 strains were lysine-decarboxylase positive, indole positive, oxidase positive, VP negative, non-haemolytic by both methods, and gave a negative chicken red-cell agglutination test. They were sensitive to Mukerjee's group-IV cholera phage and to polymyxin B. These were therefore classical strains of *V. cholerae*, serotype O-I, Inaba, and phage-type 1.

#### DISCUSSION

The isolation of lactose-fermenting *V. cholerae* strains from the recent outbreak of cholera in Calcutta is of great importance, since identification is usually done on the basis of the biochemical behaviour of a strain. Rapidly lactose fermenting vibrios may be mistaken for other organisms. The inclusion of the lysine-decarboxylase and oxidase tests will help in the identification of such organisms. All the lactose-fermenting strains were found to belong to the classical biotype of *V. cholerae* and they were all in the Inaba form.

#### SUMMARY

Fifty-seven of 131 strains of *Vibrio cholerae* that were isolated in Calcutta during an outbreak of cholera in April and May 1970 fermented lactose after overnight incubation in Andrade's peptone water and in TSI medium. All the rapid fermenters of lactose belonged to the classical biotype, to serotype O-I, Inaba, and to phage-type 1.

*V. cholerae* strains are usually non-fermenters or late fermenters of lactose. A deviation from this previously accepted finding may cause error in identification. The inclusion of the lysine-decarboxylase and oxidase tests helps to overcome this difficulty.

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