

Isolation of Mucooid *Vibrio parahaemolyticus* Strains

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Mucooid strains of *Vibrio parahaemolyticus* were isolated from the stools of two asymptomatic carriers and a patient with gastroenteritis. The strains demonstrated biochemical reactions and antibiotic susceptibility typical of nonmucooid strains of *V. parahaemolyticus* isolated locally. The slime substance was typed by coagglutination and was antigenically similar to the capsular antigen of the same strain. Three different serotypes (O10:K24, O5:K17, and O5:K15) were involved.

During the course of investigating human stool specimens for enteric pathogens on media such as 2% salted alkaline peptone water, thiosulfate citrate-bile salts-sucrose, and MacConkey and blood agars, a stool specimen produced an unusually heavy growth of mucooid colonies on blood agar (Fig. 1) and an almost pure culture of *Vibrio parahaemolyticus* on thiosulfate citrate-bile salts-sucrose simultaneously. Preliminary staining and testing with tetramethyl-*p*-phenylenediamine dihydrochloride and testing for salt tolerance showed that the mucooid growth contained gram-negative, oxidase-positive, halophilic bacilli. When the antibiotic susceptibility of the test strain was determined on Mueller-Hinton agar by the method of Bauer et al. (2), the bacterial lawn became thick and mucooid after overnight incubation, but the antibiotic susceptibility with BBL Sensi-Discs (BBL Microbiology Systems) was similar to those of normal strains of *V. parahaemolyticus* isolated locally, i.e., susceptible to kanamycin, chloramphenicol, trimethoprim-sulfamethoxazole, tetracycline, neomycin, gentamicin, and occasionally streptomycin and sulfonamide, and usually resistant to ampicillin. These observations prompted further study of the mucooid strain, which proved to be biochemically and serologically similar to the thiosulfate citrate-bile salts-sucrose culture of *V. parahaemolyticus* from the same stool specimen. Subsequently, two more mucooid strains of *V. parahaemolyticus* were isolated from stools (Table 1). Identification and serotypes of the three mucooid strains of *V. parahaemolyticus* were confirmed by Y. Takeda, Research Institute for Microbial Diseases, Osaka, Japan. He found that one strain was Kanagawa positive (Table 1).

Typing of the mucooid strains of *V. parahaemolyticus* for capsular (K) antigens was performed by direct slide agglutination with whole cells and antisera. Due to the presence of loose slime, each cell suspension was initially treated with an equal volume of 0.2 N HCl for 30 min, washed four times, and suspended in 3% NaCl solution for capsular typing. The method of Montague et al. (9) was employed for typing the somatic (O) antigens. Antisera used in the serotyping of the *V. parahaemolyticus* were produced commercially by Toshiba Kagaku Kogyo Co., Ltd., Tokyo, Japan.

The slime substance of each mucooid strain of *V. parahaemolyticus* was typed by the coagglutination method of Onokodi et al. (10). The *Staphylococcus aureus* cells (strain Cowan 1) were prepared by a modified method of Kronvall (7) as follows: the staphylococcus strain and grown in Trypticase soy broth (BBL) in a large, flat-bottomed flask at 37°C overnight. The cells were harvested, washed twice in phosphate-buffered saline (PBS) at pH 7.2, and left at room

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temperature in 0.5% Formalin-PBS for 3 h. After treatment, the formalinized cells were washed four times and adjusted to an approximately 10% cell suspension in PBS. The suspension was heated in an 80°C water bath for 20 min, washed four times, and readjusted back to a 10% cell suspension in PBS.

Antibody-coated staphylococcus cells were prepared by mixing 0.5 ml of the treated cells with a drop of K antiserum and leaving the mixture at room temperature for 30 min. The mixture was washed once and suspended in PBS to the original cell density.

The "slime" antigen was prepared by emulsifying the growth from an overnight agar culture of the mucooid *V. parahaemolyticus* strain in 2 ml of normal saline to form a thick cell suspension. The suspension was allowed to stand at room temperature for 1 h and centrifuged at 500 × *g* for 10 min. A drop of the slightly turbid supernatant (slime antigen) was mixed with a drop of the antibody-coated staphylococcus cells on a glass slide. Agglutination of the cells within 1 min was considered positive. A bacteria-free filtrate of the slime antigen was similarly tested by the coagglutination method against specific antibody-coated staphylococcus cells. Both the slime and the bacteria-free filtrate were agglutinable against staphylococcus cells coated with the same K antiserum as in the capsular typing by direct slide agglutination. These findings indicated that the slime antigen was present in the bacteria-free filtrate (3) and that the slime was antigenically similar to the capsular antigen of the same strain (6).

Two of the mucooid strains of *V. parahaemolyticus* were isolated from the normal stools of food handlers, and the

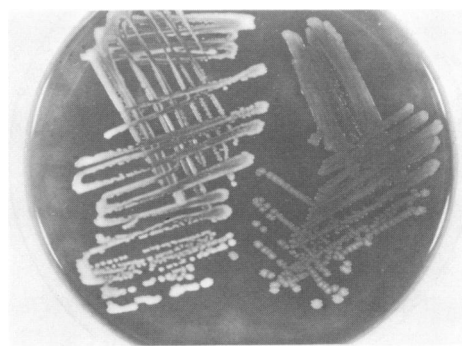


FIG. 1. Comparison of a mucooid strain of *V. parahaemolyticus* (right) with a normal strain (left) isolated from human stools. The two strains were cultured on blood agar medium and incubated at 37°C overnight.

TABLE 1. Serotyping of mucoid *V. parahaemolyticus* strains^a

Date	Strain	Sex and age	Kanagawa test	O antigen	K antigen	Source (stool)
3/22/83	Vp29.83	M, 35	–	O:10	K:24	Food handler
4/8/83	Vp38.83	M, 36	–	O:5	K:17	Food handler
4/25/83	Vp48.83	F, 32	+	O:5	K:15	Patient

^a Typing methods: (i) somatic or O antigen by direct slide agglutination; (ii) capsular or K antigen by direct slide agglutination; (iii) slime or K antigen by coagglutination. The K antigens obtained from the capsule and slime of the same mucoid strain of *V. parahaemolyticus* were identical antigenically.

third was from the diarrheal stool of a patient (Table 1). In Singapore, *V. parahaemolyticus* is a common cause of food poisoning. In the past 3 years, the annual number of diarrheal cases caused by *V. parahaemolyticus* in Singapore was over 100, and a carrier rate of 0.16% was recorded in the epidemiological report for 1982 (11) from the routine screening of public food handlers for enteric pathogens.

It has been reported that under favorable environmental conditions, mucoid colonies are sometimes cultured from nonmucoid bacteria like *Escherichia coli* (8), *Salmonella* spp. (1), *Pseudomonas aeruginosa* (4), and *Klebsiella* spp. (6). Duguid (5) reported that the degree of mucoidness depended on the amount of carbohydrate in the medium. According to Anderson (1), the production of the extracellular slime by the salmonellae was related to growth on media containing a high concentration of phosphate. However, to our knowledge, the isolation of mucoid *V. parahaemolyticus* has not been previously reported.

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