Slide Test for Detection of Slime Formation in Vibrios

Some members of Enterobacteriaceae and a few other organisms like *Pseudomonas aeruginosa* are known to produce an extracellular, viscid, amorphous surface material known as 'slime'. Phosphates and other growth promoting substances in media can enhance production of slime. ANDERSON[1] considered that the phenomenon of slime formation, which was poor or irregular on nutrient agar but abundant in presence of relatively high concentration of phosphate, was widely distributed in the family Enterobacteriaceae.

V. cholerae, except for a few mutants, were generally supposed not to produce any slime or capsule[2]. LANKFORD et al.[3] could, however, detect evidence of slime formation in the cultures of *V. cholerae*. With a modification of ANDERSON's technique, NEOGY and SANYAL[4] succeeded in demonstrating slime in several strains of *V. cholerae* and NAG vibrios, although the same could not be shown convincingly in El Tor vibrios. Working on the analogy of ANDERSON's observations, the present communication is based on a design of a simple slide technique for detection of slime formation in different vibrio species.

Material and method. Organisms: 64 strains of vibrios were studied consisting of classical *V. cholerae*, El Tor vibrios and NAG vibrios, isolated from different sources including cases of cholera. As controls, 8 members of the family Enterobacteriaceae and 2 strains of *Ps. aeruginosa* were also included. The strains were maintained on nutrient agar slants.

Medium: A special phosphate agar medium, very similar to that used by ANDERSON, was prepared with Lab. lemco (0.5%), Bacto peptone, Difco (5.0%), sodium chloride (0.85%), Bacto agar, Difco (3.0%) and phosphate buffer – pH 7.2 (q.s.). After dissolving the ingredients by heating and filtering through cotton, the pH was checked (7–7.2) and the medium was autoclaved at 15 lbs pressure for 30 min. Slants in tubes were prepared.

The surface of a clean, grease-free glass slide was divided into 2 halves by grease-pencil markings. A loopful of distilled water was put on to one half of the slide and sufficient growth from an overnight culture in phosphate agar medium was emulsified in the distilled water with a loop. The same procedure was repeated in a loopful of 0.5% sodium taurocholate solution on the other half of the glass slide. Overnight growth of the same organism in nutrient agar was tested in identical manner. Appearance of a slimy material (SM) almost immediately, in the form of a mucilaginous mass, was indicative of positive reaction. The SM not only moved along with the loop during the process of emulsification but also seemed to be adherent to it.

Results. All the 64 strains of vibrios, 2 strains of *Pseudomonas* and 2 strains of *E. coli* out of 8 Enterobacteriaceae produced SM in phosphate agar which could be detected by the slide test with both distilled water and 0.5% sodium taurocholate solution. There was, however, a difference in the degree of reaction between the strains, and within the same strain also; a stronger reaction was evident with 0.5% sodium taurocholate solution in most cases. Typical string formation, as observed by SMITH[5] in the 'String test' for vibrio identification with a stronger lysing agent – sodium desoxycholate, was noticed in vibrio strains producing copious SM. All classical *V. cholerae* produced SM in nutrient agar detectable by slide test; nevertheless, presence of phosphate in the medium definitely enhanced production of SM by these organisms. Phosphate agar medium was, however, found to be necessary for production of SM in the case of 12 out of 34 El Tor strains and 3 out of 13 NAG vibrios. Copious amounts of SM was produced by the *Pseudomonas* strains, and in one strain the surface growth in phosphate agar felt gummy during the time of removal with a loop.

Summary and conclusion. Slime production was detected by a simple slide test on a glass slide with distilled water and 0.5% sodium taurocholate solution in all the 64 strains of vibrios consisting of classical *V. cholerae*, El Tor and NAG vibrios, isolated from different sources, 2 strains of *Ps. aeruginosa* and 2 of the 8 strains of Enterobacteriaceae, when the organisms were grown in a special phosphate agar medium. Phosphate was essential for SM in many strains of El Tor and NAG vibrios. Although detectable SM was observed in nutrient agar, presence of phosphate definitely augmented its production by classical *V. cholerae*. Observation of such characteristic features in vibrios justified the consideration that the viscous material was extracellular 'slime' of the organism. This also suggests that the simple slide test as employed here can be utilized for detection of SM of intestinal bacteria after growing in medium containing suitable concentration of phosphate.

Zusammenfassung. Nachweis der Schleimbildung von Vibrionen in Enterobakterien.

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Effect of an Azo Dye on Germination and Outgrowth of *Bacillus megaterium* Spores

In previous investigations we reported on the bacteriostatic effect of some stains[1]. In the present study we wish to investigate the action of an azo dye, gentian violet, on the development of a bacterial spore into a vegetative cell, which is known to occur in 3 phases. The first, not necessarily with all spores, is activation, by which the dormant spore becomes able to germinate. The second is a catabolic process, by which spore materials are degraded.

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In complete medium, after initiation and preceding the first division, the spore enters the third phase, i.e., the phase of macromolecular synthesis (outgrowth).

Cells of *B. megaterium* (Paris strain) were grown in the following medium (A): Difco peptone, 0.1%; Difco beef extract, 0.3%; Difco yeast extract, 0.3%; manganese sulphate, 0.01%; agar, 1.5% and 1000 ml of water. After 36 h of incubation at 37 °C, spores were harvested, washed 5 times by centrifugation and examined microscopically to ascertain the purity of the preparation with regard to the presence of only spores.

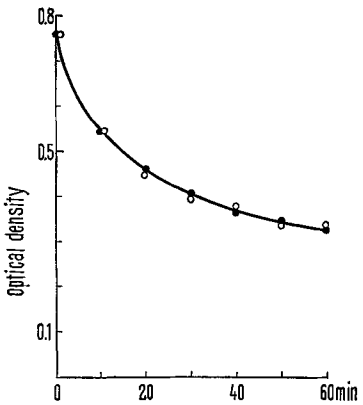

Fig. 1. Decrease in O.D. during spore germination, induced by inosine. (●—●), control; (○—○), gentian violet.

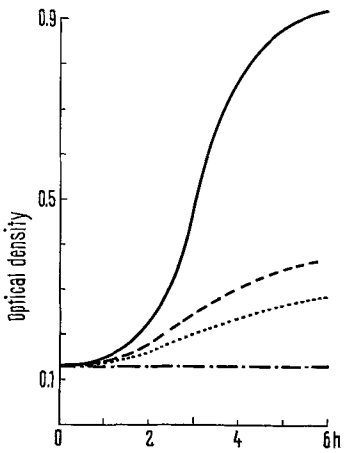

Fig. 2. The growth of *B. megaterium*. ——, control; – – –, gentian violet 0.1 μg/ml; · · · ·, gentian violet 0.4 μg/ml; –·–·–, gentian violet 1 μg/ml.

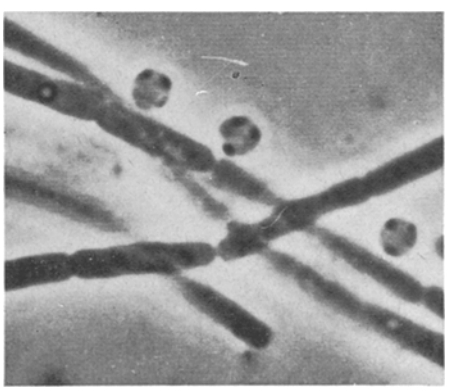

Fig. 3. *B. megaterium* vegetative forms and protoplasts obtained from a culture with gentian violet. × 6000.

Germination was judged by the decrease in absorbancy of the spore suspension. A suspension of spores (about 2×10^9/ml) in $5 \times 10^{-2} M$ phosphate buffer, pH 7.5, plus inosine ($1 \times 10^{-3} M$) displayed a 60% reduction of the initial absorbancy at 400 nm in about 60 min of incubation at 37 °C. The same fall in absorbancy may be seen when different quantities of gentian violet (from 0.10–20 μg/ml of medium) were added to the spore suspension plus inosine (Figure 1).

To investigate the effect of gentian violet on outgrowth, germinated spores were suspended in A medium and A medium with gentian violet (from 0.10–20 μg/ml of medium). Tubes were incubated at 37 °C and every half-hour, for 8 h, the absorbancy was measured.

There was no increase in absorbancy of the cultures containing one or more micrograms of azo dye per ml of medium. Small increase in absorbancy was seen when from 0.10–0.40 μg of gentian violet were added and the same rate of increase in absorbancy, as that of controls, was demonstrated at lower concentration (Figure 2). At the end of incubation cultures were examined microscopically at phase contrast and it was possible to see that when one or more micrograms of gentian violet were added to the medium, no vegetative cell can be seen. But when 0.10–0.40 μg, inclusive of stain, were added to the medium, the growth was partially inhibited and vegetative forms and protoplasts developed (Figure 3).

The protoplast formation by gentian violet raised the question of how the azo dye affects vegetative cell metabolism and division. Although it was demonstrated that gentian violet induces accumulation of nucleotide cell-wall precursors in *S. aureus*[2] and inhibiting incorporation of isotopes into cell protein and nucleic acid as well as into cell wall[3], further studies are required.

Riassunto. È dimostrato che il violetto di genziana non interferisce nella germinazione indotta con inosina di spore di *Bacillus megaterium*, mentre ne inibisce la crescita alle concentrazioni di 1 o più μg per ml di cultura. Quando impiegato a concentrazioni comprese fra 0,4 e 0,1 μg/ml è possibile mettere in evidenza non solo forme vegetative ma anche protoplasti. Quest'ultima osservazione viene messa in relazione alla attività inibente la sintesi dei precursori del cell wall da parte del violetto di genziana.

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