Use of Montmorillonite for the Demonstration of Capsules of *Klebsiella aerogenes* and *Corynebacterium* sp.

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A technique is described for the demonstration of bacterial capsules by using montmorillonite in combination with a conventional staining method.

The methods used for demonstrating the presence of bacterial capsules (1, 2, 4, 6, 7) may be grouped into positive and negative ones. In the former, the capsule itself is stained, whereas in the latter the unstained capsule appears as a clear bright zone on a darker stained background. Positive staining is usually recommended, because artifacts are liable to occur in the negative staining (8). However, because bacterial capsules differ in chemical nature, their staining properties may well be expected to vary. This is probably the reason why many research workers still prefer negative staining methods (2, 4).

During studies on the interaction between clay minerals and encapsulated bacteria, the use of montmorillonite in a combined negative-positive staining method for capsule demonstration was considered.

Encapsulated and noncapsulated strains of *Klebsiella aerogenes* [strains *K*<sub>K</sub>A and *K*<sub>54A3</sub>O], respectively, kindly provided by Professor J. P. Duguid, Department of Bacteriology, Dundee University, Scotland] were grown for 2 days at 37°C on an excess sugar medium A which allowed maximum capsule formation (3). The medium had the following composition: 2% agar (Difco), 0.2% NaCl, 0.1% K<sub>2</sub>SO<sub>4</sub>, 0.02% MgSO<sub>4</sub>7H<sub>2</sub>O, 1% CaCO<sub>3</sub>, 0.001% FeSO<sub>4</sub>, 0.1% peptone (Difco), and 1% sucrose. Strains of *K. aerogenes* were also grown on a medium B of similar salt composition but having a lower C to N ratio (0.1% sucrose and 1% peptone). In addition, a levan-forming *Corynebacterium* sp. which produces capsules when grown for 7 days at 30°C on 10% sucrose-tap water-agar (5) was examined.

The clay suspension used was a 0.5% (w/v) K<sup+</sup> montmorillonite less than 0.2 μm in size (pH 7) prepared from montmorillonite 25 (Ward's National Science Establishment, New York, N.Y.).

Bacterial capsules were stained by the method of Novelli (7) with and without the addition of equal volumes of clay suspension to the bacterial suspension before smear preparation. With this method (1 min in 1% alcoholic Alcian Blue for capsules, followed by 3 to 5 sec in carbolic fuchsin for cells), the capsules of *K. aerogenes* were stained in blue very lightly and did not show clear limits, whereas the levan capsule of *Corynebacterium* sp. did not appear after the staining. In smears containing montmorillonite, the capsules of *K. aerogenes* had greater dimensions than those prepared without the addition of clay, and capsule limits were clearly seen. Capsule size varied in the two growth media used (Fig. 1A and B). Colorless capsules of *Corynebacterium* sp. are shown in Fig. 1C. Noncapsulated bacteria [K<sub>54A3</sub>O] stained by the same procedures showed no similar structure with any of the methods used. This observation excludes the possibility that the clear limit obtained with the clay-containing smears is an artifact. The red fuchsin-stained clay particles concentrated around the capsules, thus creating a sharp boundary around the encapsulated bacteria. This may have resulted from movement of clay particles toward bacterial cells during the drying of the smear, as no such boundary was observed in wet films under a phase-contrast microscope.

The combined negative-positive method described above gives a much finer background than the India ink method (4). However, its wider use in staining methods other than that of Novelli deserves further investigation.
FIG. 1. Effect of addition of clay to bacterial smears stained by the method of Novelli (7) for demonstrating encapsulation. × 800. (a) Klebsiella aerogenes (K54A3) grown on excess sugar medium A. Clay particles stained by carbolfuchsin surround the Alcian Blue-stained bacterial capsule. (b) K. aerogenes (K54A3) grown on a sugar-deficient medium B. Capsules appear much smaller than those observed in Fig. 1A. (c) Corynebacterium sp. grown on 10% sucrose-tap water-agar; clay was added.
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