The ability to produce levan is shown by several species of bacteria in the genera *Bacillus* (Forsyth and Webley, 1949), *Aerobacter* (Aschner, Avineri-Shapiro and Hestrin, 1942), *Pseudomonas* (Fuchs, 1956), *Azotobacter* (Beijerinck, 1912) and *Corynebacterium* (Lindeberg, 1953; Henis and Aschner, 1954; Fuchs, 1959).

The number of strains of *Corynebacterium* known to produce levan is at present restricted to three and not much is known about the distribution of these bacteria in nature: Lindeberg's isolate originated from spoiled herring preserves and the other two strains were chance aerial contaminants. The purpose of this communication is to present an enrichment method for a levan synthesizing *Corynebacterium* from raw sewage or activated sludge. Some properties of the organism including factors influencing levan formation have also been considered.

**Materials and methods.**

*Isolation of the bacterium.* The enrichment medium without added nitrogen source (referred to as nitrogen-deficient medium) used for its isolation had the following composition: sucrose 10 g; CaSO₄·2H₂O, 0.1 g; MgSO₄·7H₂O, 0.2 g; NaCl, 0.2 g; K₂HPO₄, 0.2 g; Na₂MoO₄·2H₂O, 0.005 g; FeCl₃, 0.002 g; CaCO₃, 5 g; micronutrient solution, 1 ml; distilled water, 1000 ml.

The micronutrient solution contained the following ingredients in 1 L of distilled water: ZnSO₄·7H₂O, 11 g; MnSO₄·H₂O, 5 g; CoSO₄, 0.05 g; H₃BO₃, 0.05 g; CuSO₄·5H₂O, 0.007 g.
The medium was dispensed in 10 ml amounts in 50 ml Erlenmeyer flasks and sterilized by autoclaving at 121° C. for 10 min. The flasks were inoculated with 1 ml of raw sewage or activated sludge. Incubation was carried out at room temperature (25° - 28° C.). After three serial passages in the above medium, the final enrichment was plated out on the above medium solidified with 3% agar. Representative colonies were picked off and purified by the usual techniques. The organism to be described is one of several similar strains which were isolated and studied.

Characterisation of the isolates. The isolates were examined by the usual bacteriological techniques described in the "Manual of Microbiological Methods" (Edited by the Society of American Bacteriologists, 1957). Fermentation tests were performed both in the conventional peptone-water medium as well as in the newer medium of Hugh and Leifson (1953). All fermentation substrates were added in a final concentration of 1%.

Studies of the factors affecting polysaccharide synthesis. A basal medium of the following composition was used: Na₂HPO₄·12H₂O, 0.8 g; KH₂PO₄, 0.2 g; NaCl, 0.05 g; MgSO₄·7H₂O, 0.5 g; CaSO₄·2H₂O, 0.1 g; Na₂MoO₄·2H₂O, 0.005 g; FeCl₃, 0.002 g; micronutrient solution, 1 ml; distilled H₂O, 1000 ml.

Carbon and nitrogen sources were added as described below. The inoculum in all cases was a dilute suspension of aseptically washed cells usually harvested from a nutrient broth culture. Whenever necessary the bacterial population in the broth was counted by dilution to the extinction point, using nutrient broth as the growth medium. Polysaccharide formed was measured in a Klett-Summerson Colorimeter using the 540 mμ filter. In order to eliminate the turbidity caused by the cells, in all experiments a control tube was included, which contained the corresponding medium with glucose, which allows growth but no polysaccharide formation.

Preparation and purification of the polysaccharide. The organism was grown in the above basal medium with 2% sucrose. The inoculum used consisted of aseptically washed cells from nutrient broth containing initially about 10⁵ cells per ml. Incubation was carried out at room temperature for 4-6 days. The culture was mixed with four volumes of ethanol containing 0.5% CaCl₂ with constant stirring. The addition of CaCl₂ prevented the formation of a rubber-like product, difficult to pulverise. After