density determinations. Part of this work was supported by Public Health Service research grant GM 11079 from the National Institute of General Medical Sciences.

**ENCAPSULATED LACTOBACILLI**

**II. SPECIFIC CAPSULAR REACTION OF LACTOBACILLUS CASEI**

BENJAMIN F. HAMMOND, BURTON ROSAN, AND NED B. WILLIAMS

*Department of Microbiology, School of Dental Medicine, University of Pennsylvania, Philadelphia, Pennsylvania*

Received for publication 3 August 1964

Previous studies have indicated that the capsular polysaccharide of *Lactobacillus casei* plays an important role in determining many of the organisms' serological and biochemical properties (Hammond and Williams, Arch. Oral Biol. 9:341, 1964; Nature 202:929, 1964). In a survey of several hundred human saliva samples, it was observed that the specific capsular reaction (Quellung) could be used for the rapid and accurate identification of oral *L. casei* strains growing in mixed culture and producing the specific capsular polysaccharide. This report describes other uses of the reaction in the serological characterization of the organism, including the relationship between the capsular material and the cell wall.

The procedure was a modification of the indirect fluorescent-antibody technique of Weller and Coons (Proc. Soc. Exptl. Biol. Med. 86:789, 1954). A methanol-fixed smear of the test organism (*L. casei* L-324M) was exposed to an antiserum (rabbit), and after a series of rinses in 0.01 M phosphate buffer (pH 7.2) the smear was covered with a few drops of goat antirabbit globulin. The smear was washed, and was subsequently examined for changes in the refractive index and overall definition of the capsular layer by use of phase-contrast instead of fluorescence microscopy. In like manner, an unknown organism could be checked against an antiserum known to contain antcapsular antibody. Acid fuchsins, bromophenol blue, or other stains used to detect antigen-antibody reactions in immuno-diffusion studies also showed a reaction at the capsule surface but less clearly.

Figure 1 shows a control smear in which encapsulated cells were exposed to normal rabbit serum. The outlines of the cell-wall boundaries are clear, but the refractile halo around the cells merges rather imperceptibly with the background, giving only the suggestion of a capsule. Figure 2 shows cells from the same cell suspension which were exposed to specific capsular antibody obtained by immunization with whole encapsulated L-324M cells ("whole serum"). The capsular layer now appeared considerably enlarged and consisted of two layers: an inner refractile halo and a thick, dark, homogenous outer layer which frequently formed a continuous covering for groups of adjacent cells. Essentially the same picture was obtained when "whole serum" was absorbed with nonencapsulated cells, a purified cell-wall preparation (Ikawa and Snell, J. Biol. Chem. 235:1370, 1960), or various cell-wall carbohydrate extracts (Lancefield, J. Exptl. Med. 47:91, 1928; Rantz and Randall, Stanford Med. Bull. 80:391, 1955), confirming, in part, the previous report that the capsular material, although chemically related to cell wall, appears to be serologically distinct from it (Hammond and Williams, Nature 202:929, 1964). In contrast, the specific capsular reaction was completely eliminated if the serum was first absorbed with purified capsular material, encapsulated cells, or culture filtrates which contained the water-soluble capsular polysaccharide. Control slides using nonencapsulated cells against antcapsular serum and "whole serum" were consistently negative.

This cyto-serological procedure was also useful in establishing quantitative relationships. It was possible to check unknown sera for exact titers of antcapsular antibody with an encapsulated strain as test antigen against varying dilutions of the serum; the results were in good agreement with precipitin and fluorescent-anti-
body titers, but were higher than agglutinin titers. The simplicity of the test offered an easy means for judging the efficiency of various decapsulation procedures, of assaying the effect of different nutritional and cultural factors on capsule production, and of determining alterations in immunochemical specificity during any of the above experimental procedures.

This work was supported in part by Public Health Service grant DE-01956 from the National Institute of Dental Research.