NOTES

CONSTANCY OF DEOXYRIBONUCLEIC ACID BASE COMPOSITION IN THE TRANSITION OF SPHAEROPHORUS NECROPHORUS FROM BACILLI TO LARGE BODIES

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Received for publication 29 July 1964

Controversy exists concerning the relation or distinction of pleuropneumonia-like organisms (PPLO) and L-type organisms (Smith, Bacteriol. Rev. 28:97, 1964). While some feel that PPLO and L organisms may be identical, i.e., that PPLO are stabilized forms derived from bacteria (Barile et al., Oral Surg. Oral Med. Oral Pathol. 18:1395, 1963), others recognize sufficient differences to warrant placing the PPLO in the genus Mycoplasma (Klieneberger-Nobel, Pleuropneumonia-Like Organisms (PPLO Mycoplasmataceae, Academic Press, Inc., New York, 1962).

Determination of deoxyribonucleic acid (DNA) base composition of bacterial cells shows promise as an aid in microbial taxonomy (Marmur et al., Ann. Rev. Microbiol. 17:329, 1963). Little is known about the DNA base composition of L organisms or PPLO. If it could be presumed that the DNA composition of a given bacterial species remains constant throughout transition to L organisms, then DNA base ratio analyses would be useful in the characterization of L forms and PPLO. Sphaerophorus necrophorus (Bacteroides funduliformis) is one of the few bacteria which provides a model system for this transition, since it spontaneously gives rise to L forms in appropriate media. The constancy of per cent guanine plus cytosine (% G+C), as determined

FIG. 1. Photomicrographs of the Sphaerophorus cultures. (A) S. necrophorus spherical bodies, stained with Loeffler's methylene blue. (B) S. necrophorus rod form, Loeffler's methylene blue. (C) Sphaerophorus sp. L colony, unstained.
Another organism isolated as an L form from a patient and tentatively identified as a *Sphaerophorus* (Hill and Lewis, Bacteriol. Proc., p. 48, 1964) was examined for DNA \% G+C in the same manner. This organism occasionally yielded bacterial forms which were characteristic of *Sphaerophorus* by cultural and serological methods, but the culture was not stabilized in a bacterial form.

The morphological appearance of the large bodies and rod forms of *S. necrophorus*, and of the L form of the second organism, is presented in Fig. 1A, 1B, and 1C, respectively. Sketches of the densitometer tracings and density values are shown in Fig. 2. Lysates of *S. necrophorus* gave single bands in all cases. This was true for the rod forms, the large bodies and, most importantly, for a mixture of the two (Fig. 2A, B). The guanine plus cytosine content of the two forms was 31\% G+C. Two distinct bands appeared from a mixture of lysates of *S. necrophorus* and of the organism presumed to be a *Sphaerophorus* sp. (Fig. 2C, D). A value of 38\% G+C was obtained for the latter organism. It is of interest that this \% G+C value differs by 7\% from that of *S. necrophorus*.

In view of the constancy of the buoyant density of DNA from *S. necrophorus* in these two morphological states, it is proposed that determination of base composition of DNA could be usefully applied to problems concerning the relation or distinction of PPLO and L organisms. Specifically, if the various PPLO do constitute members of a single genus, the base composition of their DNA would be expected to be quite similar. On the other hand, if these organisms are identical to L forms, their \% G+C content would be heterogeneous, since L forms have been derived from bacterial genera exhibiting a broad range of DNA base composition.

It is interesting that the DNA base compositions of two *Mycoplasma* strains examined are distinctly different. Lynn and Smith (J. Bacteriol. 74:811, 1957) obtained a value of 46\% G+C for *M. hominis* 07, and Morowitz et al. (J. Mol. Biol. 4:93, 1962) observed a much lower value (33\% G+C) for *M. gallisepticum* 5069.

We are indebted to K. G. Lark, Kansas State University, Manhattan, for the use of laboratory facilities and for his assistance with the buoyant-
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

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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 anticapsular antibody. Acid fuchsin, 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 anticapsular 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 anticapsular 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-