NOTES

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

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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. **16:**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
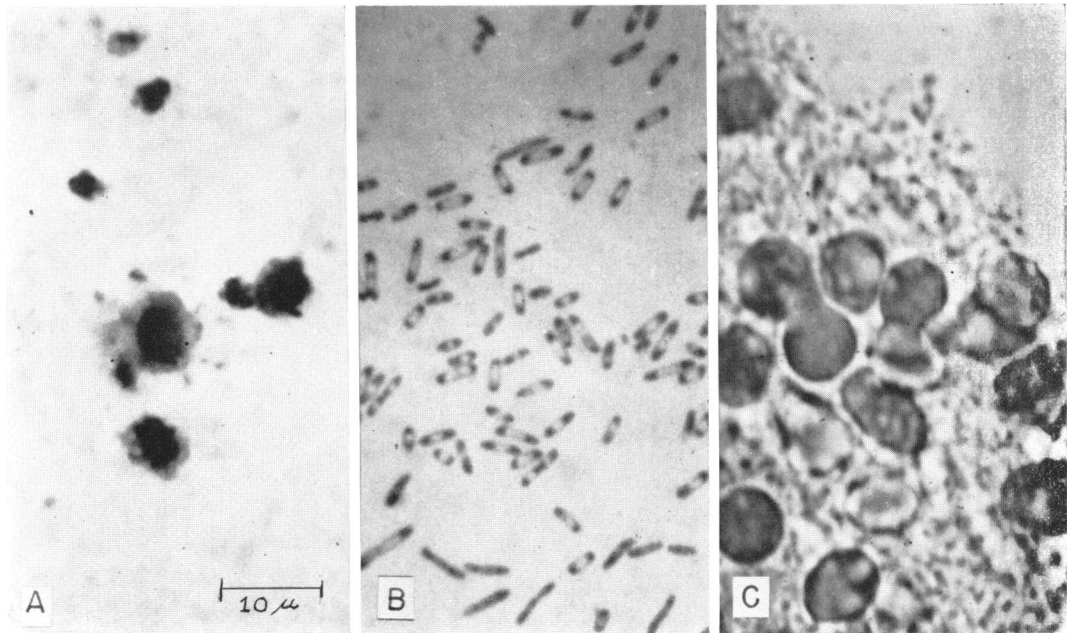

FIG. 1. *Photomicrographs of the Sphaerophorus cultures.* (A) *S. necrophorus spherical bodies, stained with Loeffler's methylene blue.* (B) *S. necrophorus rod form, Loeffler's methylene blue.* (C) *Sphaerophorus sp. L colony, unstained.*
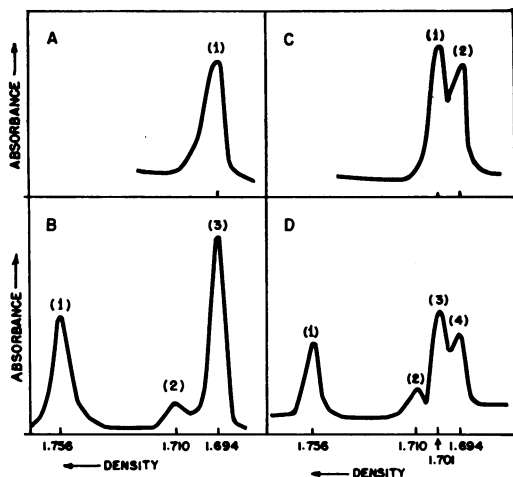
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FIG. 2. Sketches of densitometer tracings. (A-1) Single band representative of the bands obtained with lysates of the Sphaerophorus necrophorus rod form, spherical bodies, or a mixture of the two. (B-1) Bromouracil-labeled Escherichia coli DNA. (B-2) Unlabeled E. coli DNA. (B-3) Mixture of S. necrophorus rod form and spherical body lysates. (C-1, 2) Mixture of lysates of the S. necrophorus rod form and the L form of the Sphaerophorus sp. (D-1) Bromouracil-labeled E. coli DNA. (D-2) Unlabeled E. coli DNA. (D-3) Sphaerophorus sp. L-form DNA. (D-4) S. necrophorus rod form DNA.

by the buoyant-density procedure, is reported here for two diverse morphological forms of this organism.

S. necrophorus was maintained as rod forms, and as the "large bodies" of Dienes, believed to yield L forms in agar media (Dienes and Weinberger, Bacteriol. Rev. **15**:245, 1951), in continuous cultures as described by Dowell and Hill (Bacteriol. Proc., p. 29, 1964). The cultures were washed with 0.9% sodium chloride and centrifuged, and the cell pellets were frozen. After thawing, the cells were lysed with 1% Duponol. The DNA buoyant densities of cell lysates were determined after centrifugation in a Spinco model E ultracentrifuge at 42,040 rev/min for 24 hr at 25 C in approximately 5.7 M CsCl (0.7 ml of CsCl solution saturated at 70 C was cooled to room temperature and mixed with 0.33 ml of cell lysate). Bromouracil-labeled Escherichia coli DNA (ρ = 1.745 g/cc) was used as a reference standard. Buoyant densities and base compositions were calculated according to the equations of Sueoka (J. Mol. Biol. **3**:31, 1961).

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 heterogenous, 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 5969.

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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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 immunodiffusion 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 refracile 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-