JOURNAL OF BACTERIOLOGY, Nov. 1975, p. 1023-1027
Vol. 124, No. 2
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Biological Effects of Lipoteichoic Acids

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Received for publication 3 July 1975

Pneumococcal lipoteichoic acid (Forssman antigen) added to the medium of growing pneumococcal cultures caused chain formation, prevented culture lysis in the stationary phase of growth, and inhibited lysis by penicillin and by the pneumococcal bacteriophage Dp-1.

In a recent communication we reported that the pneumococcal lipoteichoic acid (Forssman antigen) is a specific inhibitor of the *N*-acetylmuramyl-L-alanine amidase of pneumococci (1). Because of the multiple physiological roles of this enzyme (3), it was of interest to examine several morphological properties of pneumococci growing in the presence of exogenous lipoteichoic acid (LTA). The inhibition of cell separation and the apparent penicillin tolerance of such cultures have already been noted in our earlier report (1); here we provide documentation of those and some additional properties of cells grown in LTA-containing medium.

Figure 1 shows chain formation of pneumococci grown in the presence of 500 μg of pneumococcal LTA per ml.

The pneumococcal LTA has no effect on the growth rate and maximum cell yield (in the stationary phase of growth) of pneumococcal cultures (1); on the other hand, 30 to 300 μg of LTA per ml in the medium inhibited the spontaneous lysis that is characteristic of late stationary-phase cultures of this bacterium (Fig. 2).

The pneumococcal LTA (1 mg/ml) could protect these bacteria against penicillin-induced lysis (Fig. 3).

Effect on phage infection. An exponentially growing pneumococcal culture (5×10^6 cells/ml) was divided into two portions; one received 1 mg of pneumococcal LTA per ml (culture A), and the second culture (B) served as control. After incubation for 2.5 h at 37 C, each culture (1 ml each) received 0.1 ml of a Dp-1 suspension containing a total of 5×10^7 plaque-forming units (PFU). After incubation at 37 C for 10 min, the cultures were centrifuged (10,000 $\times$ g, 5 min) to remove unadsorbed phage. The resuspended bacterial pellets were then assayed to determine the number of cell-associated phage PFU. Approximately 10 to 30% of the added PFU had become adsorbed in each one of the cultures. (The number of infectious centers per milliliter of the two cultures was 6 $\times 10^6$.) A portion of each phage-infected culture was further incubated at 37 C. Four hours later, culture B had lysed completely whereas culture A had remained highly turbid even after 16 h of incubation. Four hours after infection, phase contrast microscopy showed only amorphous debris in culture B whereas culture A contained mostly normal-looking pneumococci (Fig. 4). Apparently, LTA can inhibit some as yet unidentified phase of the normal phage infection cycle past the adsorption stage.

The biological effects of exogenous LTA require rather high concentrations of this material. In a previous communication we have listed several lines of evidence indicating that these biological effects are associated with the choline-containing pneumococcal F-antigen rather than with some unknown contaminating substance(s). Two additional experimental results support that conclusion: (i) all the biological effects, as well as the autolysin-inhibiting effect, could be reproduced with F-antigen preparations heated at 100 C for 2 h in 0.15 M NaCl solution (pH 7.0); and (ii) the choline-containing F-antigen preparation moved as a homogeneous material during gel electrophoresis in sodium dodecyl sulfate (Fig. 5).

Defective cell separation during cell division and resistance to antibiotic-induced lysis and stationary-phase lysis are characteristics of pneumococci in which the endogenous activity of the autolytic amidase is inhibited (3). Evidence for the possible involvement of the host autolytic enzyme in the infection cycle of the recently isolated Dp-1 phage has already been reported (2). The results described in this communication provide further support for the suggestion that one of the major physiological functions of lipoteichoic acids may be the control of bacterial autolytic enzymes.

This investigation was supported by a grant from the National Institutes of Health.

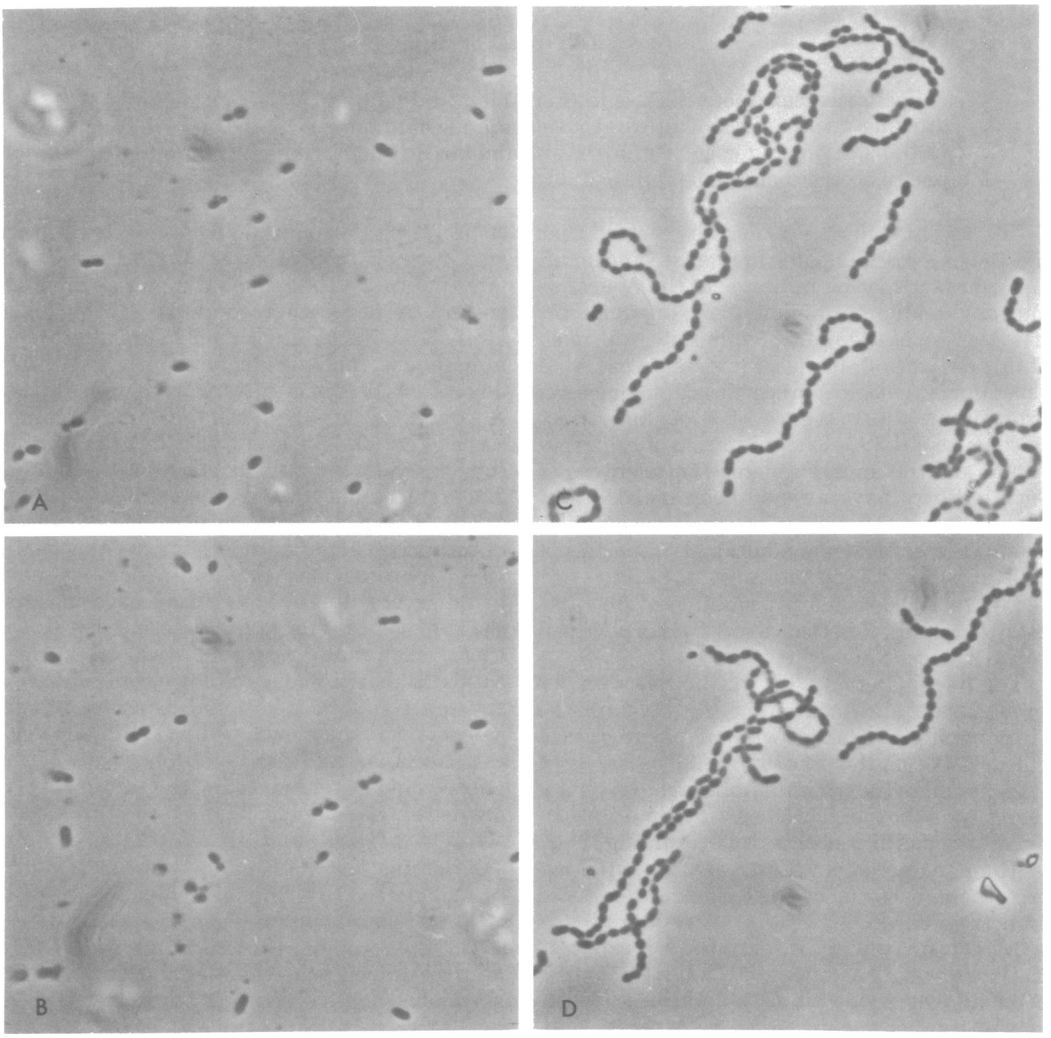

FIG. 1. *Chain formation by pneumococci grown in the presence of the pneumococcal lipoteichoic acid. An exponentially growing culture of Diplococcus pneumoniae strain R36A (2 × 10⁶ viable units/ml) was divided into two portions: one culture (2 ml) received 500 μg of purified pneumococcal Forssman antigen per ml, the other culture served as control. The preparation of Forssman antigen (1), the composition of growth medium, and the measurement of bacterial growth (3) have been described elsewhere. The extent of chaining was evaluated after 5 to 6 h of growth at 37 C. A Zeiss microscope fitted with a Planachromat 100/1.25 phase contrast oil immersion objective was used and photographs were made on Kodak Panatomic-X film. (A and B) Control culture; (C and D) culture treated with lipoteichoic acid.*

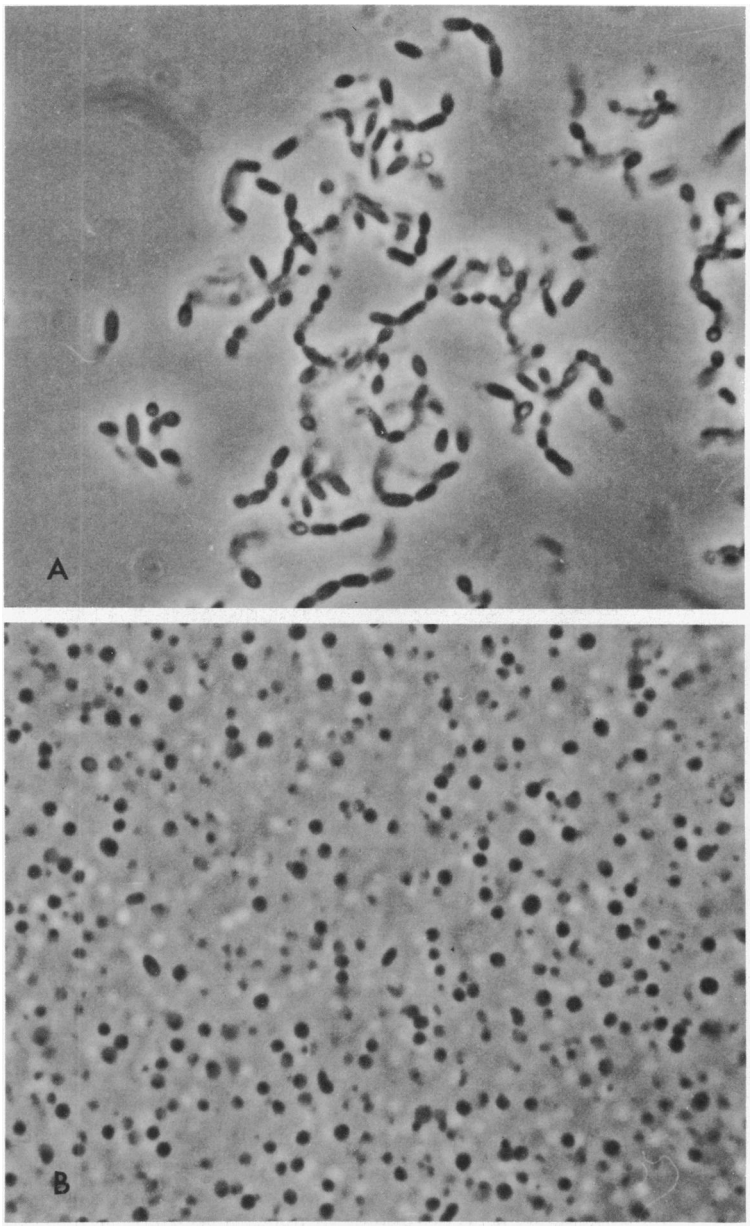

FIG. 2. *Inhibition of culture lysis in the stationary phase of growth by lipoteichoic acid. Bacteria were grown and photographed as described in the legend to Fig. 1. Photographs were taken after 24 h of incubation of the control (B) and of the lipoteichoic acid-treated (A) cultures.*

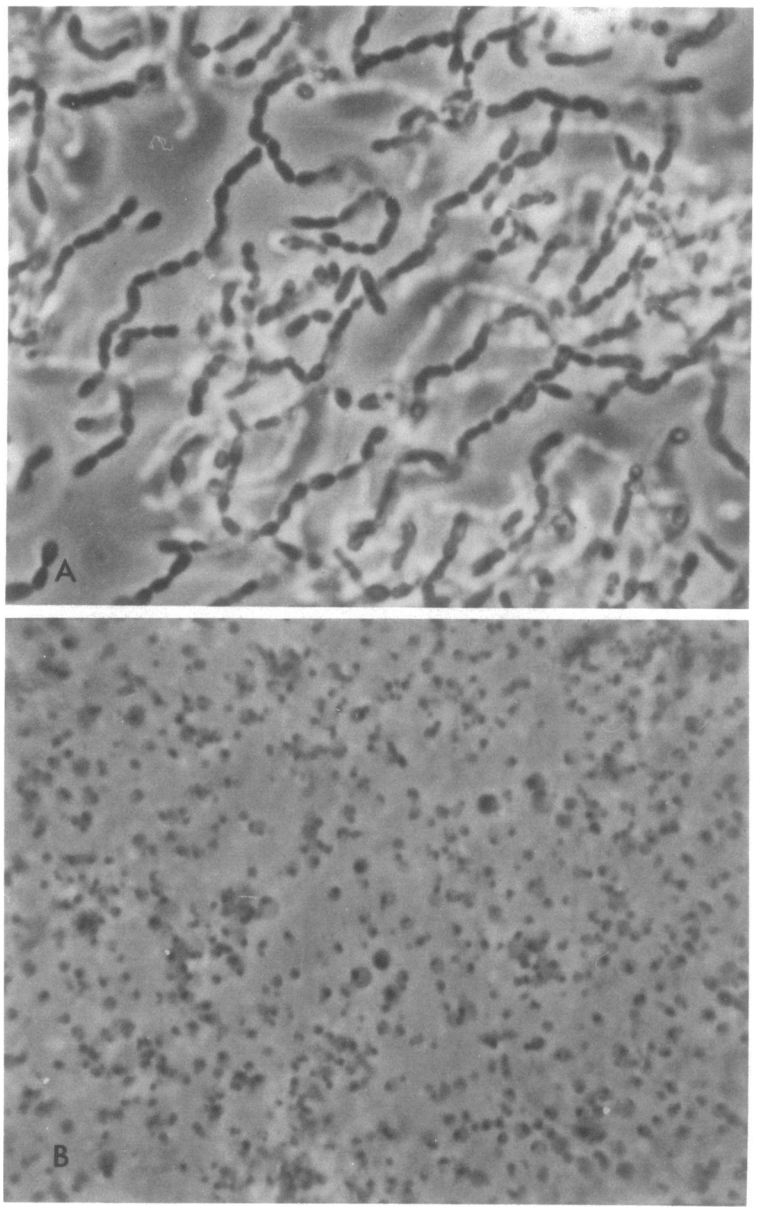

FIG. 3. *Inhibition of the penicillin-induced lysis by lipoteichoic acid. The experimental design was the same as that described in the legend to Fig. 1, except that the lipoteichoic acid (1 mg/ml) was added to one of the cultures at a cell concentration of 10^7 viable cells/ml. After incubation at 37 C for 2 h, penicillin G (0.1 U/ml) was added to both the control and the lipoteichoic acid-treated cultures. Photographs (A, lipoteichoic acid treated; B, control) were taken after 10 h of incubation with penicillin.*

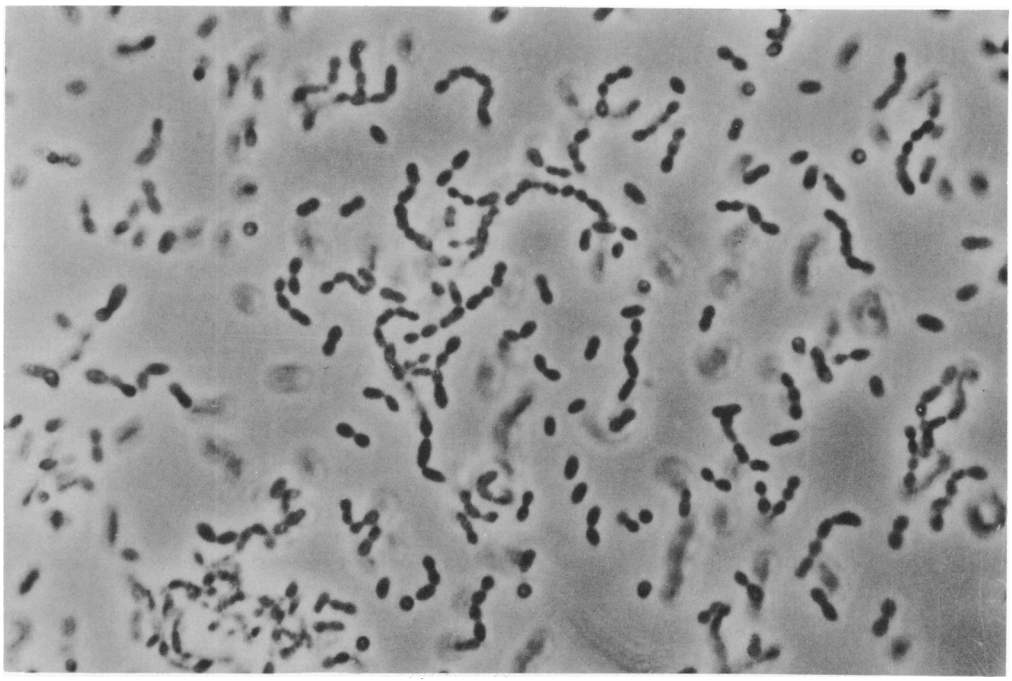

FIG. 4. *Resistance of lipoteichoic acid-treated pneumococci against lysis by the bacteriophage Dp-1. For details of the experiments, see text.*

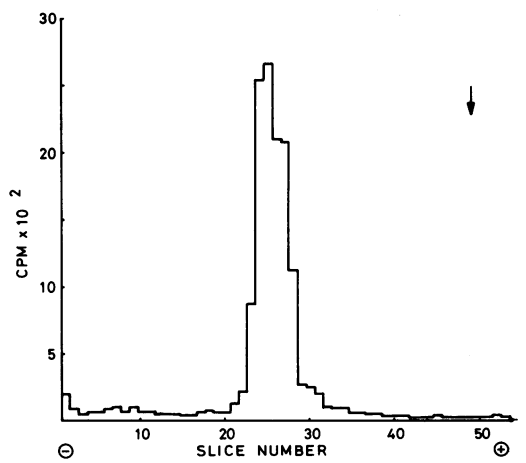

FIG. 5. *Gel electrophoresis of the pneumococcal lipoteichoic acid. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed at room temperature with 10% gels (0.5 by 9 cm) in 0.1% sodium dodecyl sulfate, pH 7.2, according to Weber and Osborn (4). The gel was cut into two equal halves. One half was stained with Coomassie blue. No bands were visible. The other half was sliced into 53 segments. The slices were digested with 0.5 ml of 90% NCS tissue solubilizer (Amersham) for 2 h at 50 C. After cooling, 10 ml of toluene-based scintillator was added and the samples were counted in a Nuclear Chicago Mark II scintillation counter. Arrow indicates position of bromophenol blue.*

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