Effects of pH on the Production of Bacterial Extracellular Drag-reducing Polymers

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High-molecular-weight, linear, soluble polymers reduce the turbulent flow frictional properties of water (1, 2). Work at this laboratory has shown the effectiveness of some microbial polysaccharides and the potential of bacteria and algae as sources of drag-reducing polymers (4, 5). The production of drag-reducing polymers in a culture medium can be demonstrated by determining the percentage drag reduction of the culture, in comparison to a water reference, in a turbulent flow rheometer (3).

During several attempts to determine the presence of drag-reducing bacteria in a water sample, the medium became acid and produced no measurable drag reduction. The addition of sodium bicarbonate, which raised the pH of the medium to about 8, often initiated drag-reducing polymer production. These results indicated that acid may inhibit drag-reducing polymer synthesis. To help determine some relationships among cell growth, pH, and synthesis of extracellular drag-reducing polymers, several pure cultures were studied.

Two freshwater isolates and one soil isolate were investigated. Isolate FITS-3, from the National Physical Laboratory tow tank, Feltham, England, was an encapsulated gram-negative rod with moist colonies. Isolate USL-2B, from the U.S. Naval Underwater Sound Laboratory water tunnel, New London, Conn., was an encapsulated gram-negative rod which produced moist to buttery colonies. Isolate HS-1, an encapsulated gram-negative rod with moist colonies, was cultured from garden soil.

The broth medium employed consisted of peptone (Difco), 0.3 g; tryptone (Difco), 0.3 g; yeast extract (Difco), 0.3 g; glucose, 3.0 g; sucrose, 3.0 g; glycerol, 3.0 ml; and deionized water, ¹ liter. All media were sterilized by autoclaving. The acid medium for isolates USL-2B and HS-1 was adjusted to about pH 4.5 with HCl, and that for FTTS-3 was left at about pH 6.5. Alkaline medium was prepared by adding 1.0 g of sterile sodium bicarbonate to 300 ml of sterile medium. A 10-ml quantity of ^a broth culture was used to inoculate 300 ml of broth in a 1-liter Erlenmeyer flask shaken at room temperature. Samples were collected aseptically at various times, and the growth, pH , and drag reduction were measured. Growth was estimated by turbidity in ^a Bausch & Lomb Spectronic-20 colorimeter at 660 nm. Drag reductions were computed from pressure drop measurements in a turbulent flow rheometer by comparing the culture sample directly to a water reference.

FIG. 1. Drag reduction by freshwater isolate FTTS-3 in relation to culture age under alkaline and acidic conditions. Both cultures were tested directly at various times for drag reduction (\Box, \blacksquare) , pH $(\triangle, \blacktriangle)$, and growth. Growth (not shown) was considerably less under acidic conditions.

Figure 1 shows the relation between pH and drag reduction for isolate FTTS-3. Growth (not shown) was considerably less under acidic conditions, with no measurable extracellular dragreducing polymer production after ⁹⁷ hr. However, under alkaline conditions, growth was more abundant, and drag reduction in excess of ¹⁵ % was obtained after 30 hr.

Isolates USL-2B and HS-1 each had similar growth rates (not shown) under acidic and alkaline conditions, except for a slight growth lag in

FIG. 2. Drag reduction by freshwater isolate USL-2B in relation to culture age under alkaline and acidic conditions. Both cultures were tested directly at various times for drag reduction (\Box, \blacksquare) , pH $(\triangle, \blacktriangle)$, and growth, Growth (not shown) at both pH values was similar.

the acid medium. The presence of drag-reducing polymers was undetected for isolate USL-2B at pH 5 after 32 hr; drag reductions in excess of 50% were obtained under alkaline conditions (Fig. 2). No drag reduction was measured in acid medium when the culture was tested further (up to 10 days). Isolate HS-1 produced approximately the same total drag reduction at pH 4.5 and pH 7.5 (Fig. 3), indicating that the acid medium did not inhibit polymer production from this isolate as it had from the other two. The drag reductions in acid medium lagged behind those in alkaline medium with culture age and can be attributed to a similar growth lag in acid medium.

When alkali-grown cultures of isolates FTTS-3 and USL-2B were acidified by the addition of HCl, drag reductions remained constant, indicating that drag-reducing polymers were not depolymerized by acid and that further polymer synthesis was inhibited. Polymer synthesis in acid-grown cultures of both bacteria could be initiated by raising the pH by the addition of sodium bicarbonate.

The importance of drag-reducing polymers to

FIG. 3. Drag reduction by soil isolate HS-1 in relation to culture age under alkaline and acidic conditions. Both cultures were tested directly at various times for drag reduction (\Box, \blacksquare) , pH $(\triangle, \blacktriangle)$, and growth. Growth (not shown) at both pH values was similar.

the hydrodynamacist is increasing, and the search for effective, low-cost polymers continues. In the isolation and screening of bacteria as possible sources of these polymers, the pH of the medium should be considered in order that some effective polymer-producing bacteria are not overlooked. Primary isolation procedures with mixed and pure cultures should include slightly alkaline buffered media to prevent possible acidification and, consequently, possible inhibition of polymer synthesis.

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