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Drag Reduction by *Acinetobacter calcoaceticus* BD4

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The encapsulated bacterium *Acinetobacter calcoaceticus* BD4 at a density of 3.6×10^9 cells per ml reduced the friction of turbulent water in a narrow pipe by 55%. This drag reduction was due to the tightly bound polysaccharide capsules (0.4 mg per ml) of culture. Capsule-deficient mutants of BD4 failed to reduce drag. The cell-bound polysaccharide demonstrated a threefold-higher drag-reducing activity than the polymer which was free in solution.

Dilute solutions of high-molecular-weight polymers greatly decrease the friction of fluids when flowing in the turbulent state. This phenomenon, initially reported almost four decades ago (9), is referred to as polymer drag reduction. Biopolymers, as well as synthetic polymers, have been shown to be effective drag reducers (2).

Acinetobacter calcoaceticus BD4 is a small gram-negative bacterium surrounded by a relatively large, tightly bound polysaccharide capsule (3). The capsule is easily visible under the microscope when a suspension of the bacteria is mixed with India ink (Fig. 1). The capsule appears white on a black background because the ink particles cannot penetrate it. Recently, the BD4 capsule polysaccharide (PS-4) was purified, and its chemical structure was elucidated (4) (Fig. 2).

The generally linear structure of PS-4, a polysaccharide with short branches, suggested that it might be an effective drag-reducing polymer. Polysaccharide PS-4 was prepared from cultures of *A. calcoaceticus* BD4 grown in glucose medium (GM) as described previously (5). Polysaccharide concentration was measured by the H_2SO_4-cysteine procedure for 6-deoxyhexoses (1) by using purified PS-4 as the standard (4).

Drag reduction measurements were performed at 30°C in a turbulent-flow rheometer similar to that described by Rosen and Cornford (7). A glass plunger driven by a constant-speed motor forces the fluid through a stainless steel tube, 25 cm long with a 1.71-mm inside diameter. The drop in static pressure of the moving fluid between two points which were 6.08 cm apart was measured directly with an electrical differential pressure transducer connected to a recorder. The rheometer was calibrated with distilled water and mercury. With distilled water, the fluid velocity in the tube was 808 cm s^{-1}, corresponding to a pipe Reynolds number of 17,200. The pressure drop for water or GM medium without glucose (M medium) was 0.395 atm (1 atm = 101.29 kPa).

The purified polysaccharide PS-4 reduced drag by a maximum of 55% at a concentration of 1.2 mg/ml (Fig. 3). Unexpectedly, an unfractionated culture of *A. calcoaceticus* BD4, containing cells and culture broth, was even more effective in drag reduction. A 55% reduction in drag was achieved with a bacterial culture (3.6×10^9 cells per ml) containing only 0.4 mg of total PS-4 per ml. Chemical analysis of bacterial cultures before and after filtration through membrane filters (0.45-μm pore size; Millipore Corp.) demonstrated that only 1.1% of the PS-4 polysaccharide was not bound to cells.

The assumption that the drag-reducing activity of *A. calcoaceticus* BD4 cultures was due to polysaccharide PS-4 was tested by analyzing the capsule-deficient mutant *A. calcoaceticus* BD4-R7. Strain BD4-R7 was derived from *A. calcoaceticus* BD4 by the introduction of a single mutation blocking PS-4 synthesis and subsequent capsule formation (5). The capsule-deficient strain BD4-R7 showed no drag-reducing ability (Table 1). Thus, the same gene was required for polysaccharide PS-4 synthesis, capsule formation, and drag reduction.

The location of the polysaccharide PS-4 responsible for drag reduction in cultures of *A. calcoaceticus* BD4 was determined by centrifugation and filtration experiments (Table 1). A culture of BD4 was diluted to 9×10^8 cells per ml and then centrifuged to yield a clear supernatant fluid and a soft pellet. The suspended pellet fraction contained over 95% of the cells and over 90% of the polysaccharide PS-4 and had a drag-reducing activity of 35% compared with an activity of 37% for the culture before centrifugation. The supernatant fluid contained only 1% of the cells and 7% of the polysaccharide PS-4 and showed a drag reduction of

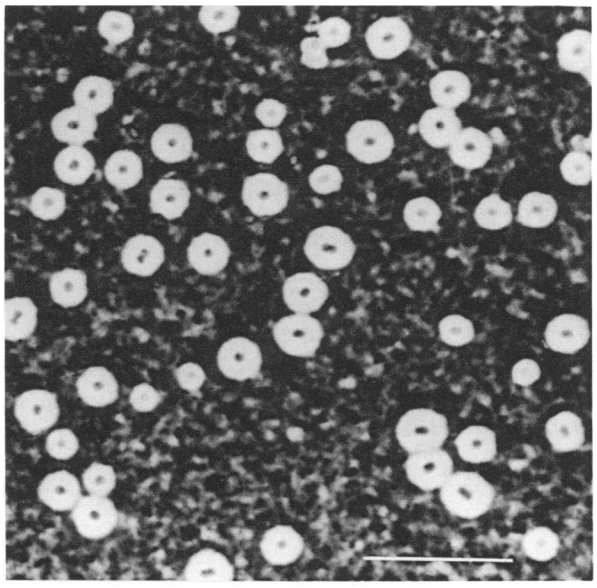

FIG. 1. Phase-contrast photomicrograph of India ink-stained *A. calcoaceticus* BD4. Bar, 10 μm.

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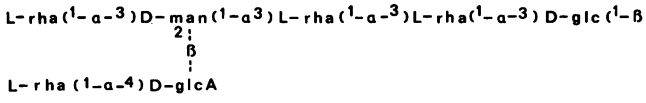

FIG. 2. Chemical structure of polysaccharide PS-4.

11%. These data demonstrate that cellular PS-4 was largely responsible for the drag-reducing activity of the *A. calcoaceticus* BD4 culture. Since the supernatant fluid of the centrifuged BD4 culture still contained 10^7 cells per ml, it was of interest to determine whether the residual 11% drag-reducing ability was due to these remaining cells or to extracellular polysaccharide PS-4. Accordingly, the supernatant fluid was passed through a 0.45-μm-pore-size filter to remove bacteria that had not sedimented during the centrifugation procedure. Control experiments indicated that less than 5% of free polysaccharide PS-4 was retained by the filter. The cell-free filtrate of the supernatant fluid contained only 1.9 μg of PS-4 per ml and had a drag-reducing activity of only 2% (Table 1). Thus, the 11% drag-reducing ability of the supernatant fluid was also largely due to cell-bound, rather than to extracellular, polysaccharide PS-4.

Drag reduction by *A. calcoaceticus* BD4 cells was not due to removal of polysaccharide PS-4 from the cells (by shear forces) during the measurement in the turbulent-flow rheometer. After passage through the rheometer, a sample was filtered to separate the cells from extracellular PS-4. The filtrate of the sample contained only 2.0 μg of PS-4 per ml and reduced drag by only 1%. Before filtration, the sample contained 105 μg of PS-4 per ml and reduced drag by 37%.

The data presented in this report provide the first demonstration that bacterial cells can reduce drag. Previously, drag reduction by bacterial and algal cultures was attributed exclusively to the production of extracellular polymers (2, 6). Our ability to show drag reduction by intact bacteria was due, at least in part, to experience with growing *A. calcoaceticus* BD4 under conditions in which it produces a large and stable capsule. When grown in the defined GM media used in this study, each *A. calcoaceticus* BD4 cell occupies a mean volume of 4.2 μm^3 and contains an average of 200,000 PS-4 molecules. A suspension of 9×10^8 cells per ml, occupying 0.04% of the volume of the fluid, reduced drag by 37%. A suspension of 10^7 cells per ml (supernatant fluid; Table 1) reduced drag by 11%. In the latter case, the average distance between cells was 0.05 mm.

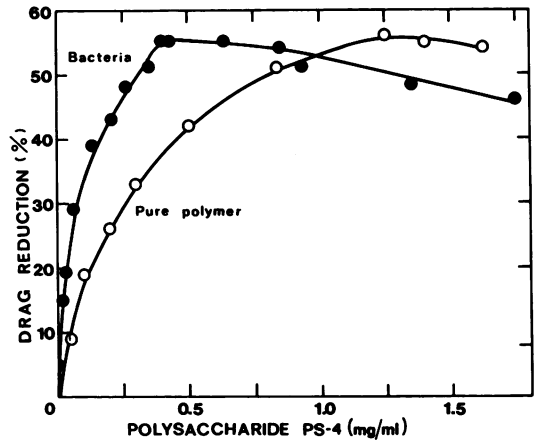

FIG. 3. Drag reduction by the capsulated bacterium *A. calcoaceticus* BD4 (●) and the purified capsule polysaccharide PS-4 (○). Drag reduction was determined with dilutions of the bacterial culture in M medium and with solutions of PS-4 in M medium.

TABLE 1. Friction reduction by *A. calcoaceticus*[a]

Source	Cell density (CFU/ml)	PS-4 concn (μg/ml)	Drag reduction (%)
BD4-R7 culture	1×10^9	3	0
BD4 culture	9×10^8	105	37
BD4 suspended pellet[b]	9×10^8	100	35
BD4 supernatant fluid[b]	1×10^7	7	11
BD4 filtrate of supernatant[c]	0	1.9	2

[a] *A. calocaceticus* BD4 and BD4-R7 were grown to late exponential phase in GM medium. Cell density, polysaccharide PS-4 concentration, and percent drag reduction were determined after diluting the cultures in M medium.

[b] The diluted culture of strain BD4 was centrifuged at 12,000 × g for 20 min at 4°C. The resulting pellet was suspended to the original volume in M medium before analyses. The supernatant fluid was analyzed directly.

[c] The supernatant fluid was filtered through a 0.45-μm-pore-size Millipore filter to remove residual cells.

For practical applications in drag reduction, cell-bound polymers may have several advantages over polymers that are free in solution. In addition to the threefold-higher activity of bound PS-4 compared with soluble PS-4 (Fig. 3), immobilized biopolymers are generally more stable than when they are free in solution (8).

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