Growth Responses of *Escherichia coli* to the Surfactant Dodecyl Benzene Sulfonate

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When strains of *Escherichia coli* are grown in broth cultures containing the anionic surfactant sodium dodecyl benzene sulfonate (NaDDBS), they exhibit unique growth responses. After 20 to 24 hr of incubation, they become slimy and viscous, and an addition of ethanol to the supernatant liquid yields a distinctive white, fibrous precipitate. The production of this material was shown to be dependent on the presence of NaDDBS in the medium. This precipitate from *E. coli* ATCC 11303 was found to contain 41 to 53% protein, 10 to 11% deoxyribonucleic acid, 6.8 to 7.4% ribonucleic acid, 15 to 25% carbohydrate, and 9% lipid. It is distinctive from naturally occurring *E. coli* slimes in several respects. Our data suggest that its formation is the primary result of the leakage of intracellular components into the medium. However, the rate of cell proliferation indicates a partial but not complete or lethal lysis. A limited utilization of NaDDBS as a carbon source was also shown.

Anionic surfactants (detergents) of the alkyl benzene sulfonate type (ABS) were reported by Anderson (2) to inhibit partially the growth of certain gram-negative bacteria in an ABS-mineral salts agar medium. In addition, certain sewage isolates grown in a 0.5% ABS-broth medium developed "a considerable amount of slimy material" after 48 hr. Growth inhibition of *Escherichia coli* by ABS varied with strains. Arkin and Anderson (Bacteriol Proc., p. 25, 1968) found a moderate inhibition of growth of cells, as evidenced by lengthened lag phase and lowered cell numbers at the stationary phase, in media containing the ABS-related sodium dodecyl benzene sulfonate (NaDDBS). Cultures grown in nutrient broth containing 0.5% NaDDBS developed marked viscosity. Addition of large volumes of ethyl alcohol yielded a heavy slimy precipitate which included a polysaccharide component (Molisch reaction). This may be taken as a general characteristic of *E. coli* since 63 different strains of this bacterium showed the formation of a similar alcohol-precipitable material (APM) when grown with this surfactant (D. A. Anderson and I. H. Koransky, Bacteriol. Proc., p. 164, 1968). Slime production was stimulated by the NaDDBS but not by the unsulfonated dodecyl benzene or the nonsurfactant sodium benzene sulfonate.

Extending these investigations, we found that with *E. coli* 11303 the APM contained not only a polysaccharide component but other fractions, indicating that the production of bacterial slime may represent a complex growth response. This paper describes some unique growth responses to NaDDBS and an examination of the APM.

**MATERIALS AND METHODS**

**Bacteria.** The bacterial strain used throughout this study was *E. coli* B (ATCC 11303) and was maintained on nutrient agar (Difco) slants. The strain grows well on a glucose minimal medium and consistently yields large amounts of APM in the presence of surfactant.

**Surfactant.** NaDDBS was obtained from Surfactant Co., Inc., Blue Island, Ill., under the trade name "Surco Bio DDBSA." Because of its high acidity (pH 1.5), it was neutralized before use to a pH 7.0 to 7.4 with concentrated NaOH solution. The surfactant solution was then added to the nutrient broth to give a final concentration of 0.5% by volume and autoclaved.

**Media.** Nutrient broth (Difco, 8 g/liter) was used as a growth medium for cells incubated with the surfactant because synthetic media gave low yields of slimy material.

The basal salts medium for the sole carbon source experiment contained: 0.2 g of MgSO₄·7H₂O, 1.0 g of K₂HPO₄, 0.5 g of FeSO₄·7H₂O, 0.2 g of CaCl₂, 0.002 g of MnCl₂·4H₂O, 0.001 g of Na₂MoO₄·2H₂O, and 10 g of NH₄Cl per liter of distilled water. The carbon sources for the experimental cells were 5.0 ml

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of NaDDBS (0.5%, v/v) per liter and glucose (0.1 g/liter) added to initiate growth. The carbon source for the control cells was glucose (10 g/liter).

Three different media were used to determine the inherent ability of the strain to produce a slime or capsular material. (A) The medium of Akashi et al. (1) contained per liter: 12 g of peptone, 0.6 g of yeast extract, 2.0 g of K2HPO4, 0.1 g of MgSO4·7H2O, and 10 g of glucose. (B) A modified Akashi medium contained per liter: 8 g of peptone, 0.6 g of yeast extract, 2.0 g of K2HPO4, 0.1 g of MgSO4·7H2O, and 20 g of glucose. (C) A medium used for the gradient plate technique (30) contained nutrient agar with 2% either of glucose or sucrose.

To detect the production of extracellular deoxyribonuclease, nutrient agar plates with 0.2% deoxyribonucleic acid (DNA; Difco) were used. Nutrient agar and DNA solutions were autoclaved separately, cooled slowly, and combined just prior to dispensing to petri plates.

**Cultural conditions.** Nonsurfactant liquid media (1 liter/2-liter Erlenmeyer flask) for the production of slime material were inoculated with a loopful of a 24-hr nutrient broth culture. Surfactant broth media (2 liters/4-liter Erlenmeyer flask) used for the production of the APM received a 10-ml 24-hr broth culture inoculum. Gradient plates were streaked with a 24-hr broth culture by using swabs. All cultures were incubated at 25°C.

**Recovery of APM.** Erlenmeyer flasks (4 liter), containing 2 liters of sterile surfactant broth and a magnetic stirring bar, were placed on magnetic stirring devices and incubated with constant stirring. The temperature of the media was kept below 35°C by the judicious use of insulation. After 1 to 4 days of incubation, 200-ml portions were centrifuged in a Sorvall refrigerated centrifuge at 13,000 × g for 30 min. The supernatant liquid was added to 2 volumes of cold 95% ethanol. The resulting APM was recovered by inserting a Nichrome wire into the mixture while stirring. This procedure effectively removed all but the very finest strands of precipitate. The precipitate was then washed three times with cold 95% ethanol, dissolved in water, lyophilized, weighed, and stored at 5°C.

**Viscosity measurements.** Measurements were made on 4.0-ml portions of APM solutions and the supernatant liquids of surfactant broth cultures in an Ostwald viscometer at 30°C before and after treatment with heat (boiling water bath for 15 min), deoxyribonuclease (20 μg in 0.1 ml), or trypsin (100 μg in 0.1 ml). The tubes with added enzymes were incubated at 30°C for 30 min to insure complete hydrolysis.

**Analytical methods.** Protein was determined by the method of Lowry et al. (19) with crystallized bovine albumin as the standard. DNA was assayed by the diphenylamine method (6) with Difco DNA as the standard. Ribonucleic acid (RNA) was determined by the orcinol method after removal of DNA (11). Total carbohydrate was determined by the anthrone method (26) by using glucose as the standard. Reducing sugar after hydrolysis (1 N HCl for 6 hr) was determined by an adaptation of the Somogy method (21) with glucose as the standard. Hexuronic acid was determined by the carbazole method of Dische (13) with glucuronic acid as the standard. Methyl pentose was assayed by the cysteine hydrochloride method (14) by using fucose as the standard.

Lipids were measured by solubility in diethyl ether. A sample (0.5 g) was suspended in 50 ml of ether, shaken vigorously, and then centrifuged at 1,000 × g for 20 min. The supernatant liquid was saved, and the pellet was washed twice with 20 ml of ether and discarded. The lipid-ether solution was evaporated to dryness and weighed. The water obtained by lyophilization of APM was determined by drying at 105°C to constant weight (2 hr). Bacteria were counted by the standard plate count method.

**Preparation of cells for analysis.** After 1, 2, 3, and 4 days of incubation, cultures grown with and without surfactant were centrifuged at 13,000 × g for 30 min in the cold. The pellets were recovered and disrupted by sonic treatment (Branson, Instruments, Inc., Stamford, Conn.). The sonically treated cells were lyophilized, weighed, and assayed for protein, carbohydrate, and DNA. The supernatant liquids from the surfactant broth cultures were harvested for the APM, which was then assayed in the same manner. No APM was obtained by adding the supernatant liquids of nutrient broth cultures (without surfactant) to ethanol.

**Production of capsular material.** An attempt was made to stimulate normal production of capsular or slime material (without surfactant) in medium A and B. After 24 and 48 hr, the cultures were examined microscopically for encapsulated cells by the wet-film India ink method (15). The cultures were also centrifuged to remove the cells, and the supernatant liquids were added to 2 volumes of 95% ethanol to determine the presence of APM. The gradient plates were examined after 24, 48, and 96 hr for smooth or mucoid colonies (20).

**Detection of extracellular deoxyribonuclease.** The production of an extracellular deoxyribonuclease by this strain was tested by a modified Jefferies et al. (17) method. Nutrient agar plates containing DNA (0.2%) were inoculated by stabbing with a straight inoculating needle. After 48 hr of incubation, the plates were flooded with 5% trichloroacetic acid. Clear zones around the colonies indicate deoxyribonuclease production.

**RESULTS**

**Utilization of the surfactant.** *E. coli* 11303 was capable of cell proliferation in a medium which contained NaDDBS as the sole source of carbon (Fig. 1). Cells grown in NaDDBS significantly showed a longer lag phase than that of the control, as evidenced by the viable cell counts (2 hr) and optical density (OD) readings (4 hr). The stationary phase was reached in the glucose medium after 14 hr, whereas that of the NaDDBS cultures was about 18 hr. The frequency and number of cell divisions for the cells in NaDDBS was lower than the control.
The addition of the supernatant liquid or the whole culture of cells grown without surfactant to 2 volumes of 95% ethanol did not yield a precipitate. The addition of the supernatant liquids of surfactant broth cultures to alcohol yielded a stringy white precipitate.

**Composition of the APM.** Chemical analyses of lyophilized samples of APM showed that its composition of protein, carbohydrate, and DNA varied over an incubation period of 96 hr (Fig. 3). A number of analyses of samples (Table 1) from 48-hr cultures in surfactant showed a mean composition of 45.8% protein, 10.6% DNA, 7.1% RNA, 17.9% carbohydrate, and 9.4% ether-soluble material. No separation or purification of the components was made prior to analysis. Consequently, assays for hexuronic acid by the carboxazole method were inconclusive, and the value obtained for methyl pentose assayed by the cysteine hydrochloride method may be suspect.

**Viscosity measurements.** Solutions of APM showed a significant decrease (Table 2) in viscosity after heat and deoxyribonuclease treatment, as did the control solutions of DNA. Trypsin treatment had only a slight effect. Heat and deoxyribonuclease treatment effected a significant decrease in the viscosity of the cell-free supernatant liquids of NaDDBS cultures (Table 3). Trypsin produced little effect. This indicated that the viscosities of the APM solution and the supernatant liquids of surfactant broth cultures can be attributed primarily to DNA.

**Analysis of cells and APM.** Cells were removed by centrifugation from 1- to 4-day nutrient broth cultures with and without surfactant.
and analyzed for protein, carbohydrate, DNA, and APM (Table 4). In 2-liter cultures, the total protein in the surfactant-grown cells, plus that in the APM, approximated that in the control cells. This apparent conservation of protein persisted over all 4 days, suggesting that the protein lost from the experimental cells was found in the APM. In terms of total carbohydrate and DNA, the APM contained greater amounts of these than the combined contents of experimental and control cells. The observation of greater quantities of carbohydrate and DNA in the APM than in both groups of cells suggests either a net synthesis of these components in response to the surfactant or the inability of the experimental cells to reutilize the carbo-
TABLE 4. Total protein, carbohydrate, and DNA of cells and APM (experimental) grown in the presence of NaDDBS and cells (control) in the absence of NaDDBS

| Component | Incubation period (days) | Experimental | Control |
|-----------|-------------------------|--------------|---------|
|           | Cells (mg) | APM (mg) | Total (mg) | Cells (mg) |
| Protein   | 1 | 160 | 71 | 231 | 240 |
|           | 2 | 212 | 140 | 352 | 355 |
|           | 3 | 202 | 141 | 343 | 352 |
|           | 4 | 198 | 139 | 337 | 362 |
| Carbohydrate | 1 | 9.38 | 21.4 | 30.3 | 9.91 |
|           | 2 | 13.4 | 35.1 | 48.5 | 13.0 |
|           | 3 | 12.9 | 35.9 | 48.8 | 11.5 |
|           | 4 | 11.0 | 36.9 | 47.9 | 14.5 |
| DNA       | 1 | 15.0 | 15.2 | 30.2 | 8.20 |
|           | 2 | 19.5 | 31.4 | 50.9 | 12.2 |
|           | 3 | 16.8 | 36.0 | 52.8 | 8.44 |
|           | 4 | 16.4 | 36.2 | 52.6 | 9.10 |

* Grown in 2 liters of broth culture. APM, alcohol-precipitable material; NaDDBS, sodium dodecyl benzene sulfonate.

growth was noted. However, 11 smooth colonies were found after 48 and 96 hr on plates containing glucose. These were located at the highest concentration of sugar (2%) on the plates, indicating that a minute portion of the population is capable of the production of capsular or slime material under suitable conditions on solid, but not in liquid, media.

DISCUSSION

This investigation indicates that E. coli 11303 is capable of utilization of the surfactant NaDDBS as the sole source of carbon. If, as has been suggested (5, 27, 29), the alkyl portion of the NaDDBS molecule is broken down by the enzymes responsible for the hydrolysis of fatty acids (β-oxidation), our results are in agreement with those workers who have found that the degradative system for fatty acids is functional and inducible in E. coli (24, 31).

E. coli is capable of growing in a complex medium, such as nutrient broth, in the presence of 0.5% NaDDBS and the growth (cell division) was shown to be partially inhibited by the surfactant. A loss of OD for the cells in the surfactant medium was noted after 14 to 15 hr, without a concomitant drop in the viable cell count. If OD readings may be assumed to be roughly proportional to the amounts of intracellular material, this decline suggests a loss of intracellular constituents. The known disruptive effect of anionic surfactants on the osmotic function of bacterial cells (22) tends to support our view that one of the effects of NaDDBS in this system is to cause the leakage of intracellular components into the medium (12).

The appearance of APM was found to occur 20 to 24 hr after inoculation into nutrient broth containing 0.5% NaDDBS. Microscopic examination of the cultures failed to show encapsulated cells. The APM may thus be more appropriately termed a bacterial slime, since many

TABLE 5. Production of capsular or slime material by E. coli strain 11303

| Incubation period (hr) | Medium A | Medium B |
|------------------------|----------|----------|
| 24                     | No capsules* | No capsules |
|                        | No APM*    | No APM   |
| 48                     | No capsules | No capsules |
|                        | No APM     | No APM   |
| 96                     | No capsules | No capsules |
|                        | No APM     | No APM   |

* Broth culture examined for encapsulated cells by wet-film India ink method.
* Supernatant liquid from culture added to 2 volumes of 95% ethanol and noted for appearance of alcohol-precipitable material (APM).
cells were seen to be embedded in large masses of this material. The production of a surfactant-stimulated slime has been reported (37; V. Arkin and D. A. Anderson, Bacteriol Proc., p. 25, 1968) in a different strain of *E. coli* although the material was not characterized chemically. The evidence presented herein, however, seems to indicate that the APM from surfactant broth cultures may not be classified as a "typical" *E. coli* slime for the following reasons. (i) The extracellular APM contains a relatively high nucleic acid content (10.0 to 11.3% DNA, 6.8 to 7.4% RNA). Such high levels of nucleic acid have not been reported in detailed analyses of the slime or capsular material of *E. coli* (1, 3, 4, 18, 25). (ii) The viscosity of liquid cultures and aqueous solutions of slime and capsular material of *E. coli* is due to the polysaccharide component (1, 25). The viscosity of surfactant broth cultures and aqueous solutions of APM is attributed here to the DNA component. (iii) The viscosity of solutions of APM is greatly decreased by heat treatment, whereas the polysaccharide of *E. coli* (18) is unaffected by treatment in the same manner. (iv) This strain (ATCC 11303) does not form slime in liquid media designed for its production.

In spite of the arguments presented above, the possibility of the production of a high-molecular-weight polysaccharide in response to NaDDBS cannot be disregarded, since a significant polysaccharide component (15.9 to 25.0%) was detected. Chemical analysis demonstrated the presence of a small quantity (0.26%) of methyl pentose. The presence of methyl pentose (fucose) in the extracellular polysaccharide material of a bacterium was first shown by Norris et al. (23) with *Lactobacillus bifidus* and has been taken as an indicator of slime material by Wilkinson et al. (32) and Kang and Markovitz (18).

Further, nucleic acids have been found as extracellular material in some bacteria. Certain halophilic organisms (*Micrococcus halodenitrificans, Vibrio costiculus, and Pseudomonas salinaria*) produce a viscous extracellular material, consisting predominately of DNA, when incubated in suboptimal concentrations of salt (28). Campbell et al. (7) observed a DNA content of 80% in cultures of *Micrococcus sodonensis*. Catlin and Cunningham (10) noted the accumulation of extracellular DNA by various non-halophilic bacteria, including *Micrococcus, Staphylococcus*, and *P. aeruginosa*. The same results have been reported (9) for *P. fluorescens, Alcaligenes faecalis, S. aureus, and S. epidermidis*. The extracellular accumulation of DNA was demonstrated under conditions which were designed to minimize extracellular deoxyribonuclease activity (19). Since this strain of *E. coli* failed to produce an extracellular deoxyribonuclease, the accumulation of DNA shown in the parallel analyses of cells and AMP from surfactant broth cultures may be explained. Eagon (16) found that the extracellular slime of *P. aeruginosa* strain 64 contained mannose (41 to 62%), DNA (10 to 12%), RNA (3 to 10%), and protein (2 to 3%). Carson and Eagon (8) found that the extracellular material from the same bacterial strain contained DNA which arose spontaneously from an intracellular origin owing to cellular disintegration. They concluded that the production of a DNA slime and polysaccharide (a mannan) were two individual phenomena. We suggest that the extracellular APM from *E. coli* cultures incubated with surfactant is likewise a consequence of two separate phenomena: portions of this material result from the leakage of intracellular components (DNA, RNA, and protein) due to the action of the surfactant, and the high-molecular-weight polysaccharide may be a synthetic result of the breakdown of portions of the surfactant molecule.

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