Possible Involvement of the Division Cycle in Dispersal of
Escherichia coli from Biofilms

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Growth rate control of adherent, sessile populations was achieved by the controlled perfusion of membrane-associated bacterial biofilms by the method of Gilbert et al. (P. Gilbert, D. G. Allison, D. J. Evans, P. S. Handley, and M. R. W. Brown, Appl. Environ. Microbiol. 55:1308–1311, 1989). Changes in cell surface hydrophobicity were evaluated with respect to growth rate for such sessile Escherichia coli cells and compared with those of suspended (planktonic) populations grown in a chemostat. Newly formed daughter cells shed at the various growth rates from the biofilm during its growth and development were also included in the study. Surface hydrophobicity decreased with growth rate similarly for both planktonic and sessile E. coli; no significant differences were noted between the two. Daughter cells dislodged from the biofilm, however, were significantly more hydrophilic than those remaining, indicating that hydrophobicity changed during the division cycle. Our data support the hypothesis that dispersal of cells from adhesive biofilms and recolonization of new surfaces reflect cell-cycle-mediated events.

Direct biochemical evidence has demonstrated that in nature bacteria grow mainly as adherent microcolonies enveloped within extensive polysaccharide matrices (4, 8). Biofilm formation occurs on virtually any surface in any environment in which bacteria are present. These include natural (3) and industrial (9) ecosystems and infections (8, 10, 11, 19). From an ecological standpoint, growth as a biofilm offers survival advantages in bacterial pathogenicity (6, 8). Cells detach from existing adherent populations and colonize new surfaces; the need to survive within the host is associated with this spread of infection. As such, bacterial biofilms have been found to be extremely persistent when confronted with host-mediated phagocytic ingestion (8) and aggressive antibiotic treatment (21). The availability of a solid substratum also promotes the accessibility of essential nutrients in an otherwise nutritionally unfavorable environment (16). In bacterial infection, hydrophobicity measurements are therefore a nonspecific method of assessing changes in cell-surface properties (1). Such changes in surface hydrophobicity might result from compositional and/or structural changes in more than one surface component.

The nature of the growth environment has a profound influence on the regulatory mechanisms determining surface properties of bacteria. Indeed, the gram-negative cell envelope shows remarkable flexibility in both structure and composition in response to changes in the surrounding environment (6, 7, 14). Whereas phenotypic variation associated with growth rate and nutrient deprivation has been well documented, the influence of growth rate within adhesive biofilms has been difficult to model and is therefore less well understood. The properties of adherent bacterial populations have been modeled through the use of a device where cells attach to removable pistons that form part of the growth chamber (20). Although providing much valuable data, this technique lacks effective growth rate control and therefore does not differentiate between properties attributable to growth rate and those associated with adhesion (5).

The approach in the present study utilized a novel, in vitro method, which, through the application of appropriate nutrient limitation in a modified chemostat, enables growth rate control of adherent bacterial populations (17). Escherichia coli ATCC 8739 was cultivated in carbon-limited, chemically defined simple-salts liquid medium (CDM) at 37°C (2). Biofilms were established, by pressure filtration, on one side of a cellulose acetate membrane support and perfused with sterile medium through the membrane and biofilms. Rates of perfusion control growth rate of the adherent population at steady state (17). Cells eluted from the biofilm together with partially spent medium are at an early stage of the division cycle and correspond to newly formed daughter cells (17). Cell-surface hydrophobicity was determined for chemostat-grown daughters, daughter cells eluted from the biofilm, and also cells resuspended from the biofilms by hydrophobic interaction chromatography (HIC) as described by Smyth et al. (24). This employed octyl-Sepharose 4B as the nonpolar ligand. HIC provides a rapid and convenient method of measuring surface hydrophobicity and is not prone to dehydration effects associated with contact angle measurements.

Figure 1 illustrates the changes in cell surface hydrophobicity during a representative batch culture of E. coli in liquid CDM (100 ml in 250-ml Erlenmeyer flasks, 37°C, 150 rpm). The inoculum was from a late-stationary-phase culture grown under the same conditions. From the lag phase to the late-exponential growth phase, cells decreased in surface hydrophobicity. Upon the onset of the stationary phase however, surface hydrophobicity increased dramatically over a period of approximately three generations. Thereafter, a gradual decrease was observed (Fig. 1). These data are in contrast to those reported for Staphylococcus aureus (13), in which log-phase cells increased in surface hydrophobicity compared with those in the stationary phase.

The effect of growth rate upon cell surface hydrophobicity in unattached planktonic populations was assessed by using chemostat cultures. Cells were removed from the fermentor (18) at various steady-state dilution rates and diluted to give a fixed cell concentration (5 × 10⁶ cells ml⁻¹) before surface hydrophobicity was measured. Results were compared with those of resuspended biofilm populations grown at identical

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growth rates. These suspensions were obtained by vortexing the cellulose acetate support membranes to remove and disperse adherent cells (Fig. 2). The growth rate of the sessile populations was confirmed by performing viable counts upon the eluates, containing newly formed daughter cells, and the resuspended biofilm populations. Since steady-state has been demonstrated in both of these populations (17), the growth rate could be calculated from a known elution rate. No significant differences in surface hydrophobicity existed for cells grown either planktonically or as biofilms. Both the biofilm populations and their planktonic counterparts became less hydrophobic as the specific growth rate was increased. In this respect, the results reflected those in batch culture (Fig. 1). The observed difference in the maximum hydrophobicity values obtained for batch and continuous cultures occurred as a result of the number of cell generations at a particular growth rate. Whereas biofilm and chemostat cultures spent at least five generations at each growth rate, batch culture cells were restricted to one generation at most. The profound influence of growth rate upon bacterial surface hydrophobicity has been previously described (13, 22, 25); for Staphylococcus, Pseudomonas, Alkaligenes, and Arthrobacter species, hydrophobicity increased with growth rate. In those studies, however, hydrophobicity was determined either by the BATH technique (23) or by contact angle measurements (13). The possibility existed that HIC did not produce results that were comparable to those (26). Considerable variation in relative surface hydrophobicity has been described; the variation is dependent upon the specific method of determination (12, 15). HIC was therefore used to assess surface hydrophobicity of mucoid Pseudomonas aeruginosa cells grown in the chemostat (data not shown). There was an increase in hydrophobicity with growth rate, which was in general agreement with other publications (13, 25). Our findings, therefore, suggest not only that some envelope properties attributed to the biofilm mode of growth are growth rate related, but also that the effect of such changes upon surface hydrophobicity varies between species.

Although hydrophobicity altered dramatically with growth rate for the chemostat and biofilm E. coli, little change with respect to growth rate was observed for the hydrophilic newly formed daughter cells freshly eluted from the biofilm (Fig. 2). The relative hydrophilicity of the daughter cells was maintained for some time after elution but, after incubation at 37°C for 1 to 2 h, increased to approach that of late-logarithmic-phase batch cultures. These results indicate that changes in surface hydrophobicity may be associated with the division cycle and that surface hydrophobicity at and immediately after division for E. coli is low. Thus, bacteria dislodged from these biofilms provided an inoculum of cells capable of replication and the colonization of new surfaces both in vivo and in the general environment. Increases in hydrophilicity associated with division processes correlated with the changes in surface hydrophobicity observed during the logarithmic phase of batch culture (Fig. 1), which would

FIG. 1. Changes in surface hydrophobicity of E. coli along the growth curve (t, 60 min). Surface hydrophobicity, as measured by HIC with octyl-Sepharose 4B, was expressed in terms of percentage of retention to hydrophobic ligands in the presence of 4 M NaCl.

FIG. 2. E. coli surface hydrophobicity as a function of growth rate (C limitation). HIC measurements were performed on populations of (○) sessile cells, (•) planktonic cells, and (■) biofilm eluates (newly formed daughter cells).
presumably relate to an increased proportion of newly divided cells.

It is tempting to suggest that similar mechanisms might apply more generally. However, the opposite trends of surface hydrophobicity and growth rate observed for other cell types suggest the following: (i) that surface hydrophobicity reflects alterations of the cell surface, possibly with respect to surface appendages or proteins associated with adhesion (22); (ii) that such adhesive structures are minimized during and immediately after the division period, leading to daughter cell separation and dispersal; and (iii) that such changes in adhesiveness do not always covary with surface hydrophobicity (27). Our data support the hypothesis that dispersal of cells from adhesive biofilms and recolonization of new surfaces reflects cell-cycle-mediated events.

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LITERATURE CITED


