Effect of ColV plasmids on the hydrophobicity of Escherichia coli

(Hydrophobicity; sex pili; ColV+, E. coli)

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1. SUMMARY

The hydrophobicity of E. coli strains carrying or lacking the colicin V (ColV) plasmids, ColV, I-K94 or ColV-K30 was assayed. ColV + derivatives of strain 1829, produced by conjugation or transformation, were more hydrophobic than either the original 1829 parental strain or a Col- derivative formed by curing 1829 ColV-K30 of its plasmid by an SDS/high temperature growth technique. Transfer of ColV into other E. coli strains also led to increased hydrophobicity. This effect of ColV plasmids was observed for organisms grown at 37°C; ColV- strains did not differ in hydrophobicity if grown at 21°C. This finding and other studies suggest that sex pili may be involved in the increased hydrophobicity.

2. INTRODUCTION

The presence of ColV plasmids in Escherichia coli strains enhances their virulence in experimental animals in comparison with plasmid-free strains [1].

The colicin V itself is not responsible for increased virulence since sterile preparations of this colicin are not lethal for animals and ColV plasmids unable to produce colicin still increase virulence [1]. The enhanced virulence of ColV + strains of E. coli has been attributed to various factors. First, ColV + strains survive better in serum than do strains lacking ColV [2]. Second, some ColV plasmids have been found to encode a novel iron-chelation system [3,4]. Third, ColV + strains adhere better to mouse intestinal epithelium than the isogenic strains lacking this plasmid [5]. It is also possible that the presence of the ColV-encoded major outer membrane protein, VmpA protein, is involved in virulence [6,7]. The ColV plasmids used here are ColV-K30 and ColV, I = K94. Both enhance the virulence of E. coli strains which carry them [2]. These two plasmids are conjugative and derepressed in transfer properties; their transfer properties and pili closely resemble those of other derepressed F-like plasmids [8].

Our study of these plasmids establishes yet another ColV phenotype: E. coli strains possessing either of the ColV plasmids are more hydrophobic than their ColV-negative counterparts possibly due to the effects of sex pili.

3. MATERIALS AND METHODS

3.1. Bacterial strains

All the strains used in the present study are E. coli K12 derivatives. The origins of strains 1829 trp, P678-54 thr, leu, thi and 1157 thr, leu, pro, his, arg, thi have been described [6,7,9]. The deriva-
tives carrying ColV-K30, ColV-I-K94, Flac, R124 and ColV, I-K94 plus R124 were made by conjugal transfer [9]. A derivative of strain 1829 carrying ColV, I-K94 produced by transformation with ColV, I-K94 DNA was kindly provided by C.M. Deeney. The strains were maintained on Oxoid nutrient agar (NA) at 4°C and subcultured at 2-month intervals on NA.

3.2. Growth conditions and bacterial suspensions

E. coli strains were grown in 25 ml Oxoid nutrient-broth (NB) overnight at 37°C or 21°C without shaking. The cells were centrifuged, washed once and then gently suspended in 0.01 M phosphate-buffer (pH 7.0) to give a thick suspension (approx. $1 \times 10^{10}$ cells/ml) for hydrophobic interaction chromatography (HIC, see below).

For growth in minimal medium, static cultures in such medium [6] supplemented as appropriate with required L-amino acids and thiamine at 20 $\mu$g/ml were used. Cells were prepared for HIC (below) as described for those grown in broth.

For studies of pilus retraction with cyanide, organisms were incubated as described by Novotony and Fives-Taylor [10] for various periods with $10^{-2}$ M cyanide at 37°C, washed and prepared for HIC (see below).

3.3. Curing of ColV plasmid

E. coli 1829 ColV-K30 was inoculated into NB containing 1% SDS and incubated at 37°C for 48 h without shaking. One ml of the culture-broth was transferred to 10 ml fresh NB and incubated for another 48 h at 44°C. The culture was then inoculated onto the NA plates and colonies tested for the absence of colicin V [9].

3.4. Re-introduction of ColV-K30 into a ColV- strain

Strain 1829 cured of ColV-K30 was mixed with strain 1157 ColV-K30 in broth for 2 h at 37°C. The mixed culture was then plated on synthetic medium (g/l: KH$_2$PO$_4$12, K$_2$HPO$_4$ 28, sodium citrate 2, MgSO$_4$7H$_2$O 0.4, ammonium sulphate 4.0, glucose 2.0 and tryptophan 0.02). The colonies that grew on this medium were tested for colicin production.

3.5. Colicin test

Overnight grown cultures of E. coli were streaked on NA plates and incubated at 37°C for 24 h. After exposure to chloroform vapour for 15 min and then exposure to air for 30 min the plates were overlaid with 5 ml of soft-agar (g/250 ml: Oxoid No. 2 broth 2.5), MgCl$_2$ 0.5, NaCl 0.75, Agar (Oxoid) 1.8) containing 100 $\mu$l (approx. 10$^8$ organisms) of an overnight broth culture of strain 1829 grown at 37°C. The plates were incubated at 37°C for 24 h and observed for zones of clearing around the streaked cultures.

3.6. Shearing

The ColV$^+$ and ColV$^-$ strains of E. coli 1829 were grown overnight in NB at 37°C. The cultures were blended for 2 min in a blender maintained at 4°C. The cells were centrifuged, washed once with 0.01 M phosphate-buffer (pH 7.0) and suspended in a small volume of the same buffer.

3.7. Hydrophobic interaction chromatography (HIC)

The retention of bacteria on octyl-Sepharose (Sigma) was determined in the presence of various concentrations of ammonium sulphate [11]. Pasteur pipettes were plugged with glass-wool and packed with octyl-Sepharose (CL 4B) to a bed volume of 1.0 ml of Sepharose. The columns were equilibrated with required molarities of ammonium sulphate in 0.01 M phosphate buffer (pH 7.0). To such columns 100 $\mu$l aliquots of the E. coli suspensions (washed and suspended in phosphate buffer) were applied and eluted with 4.0 ml phosphate buffer containing the concentration of ammonium sulphate to which the column was equilibrated. The absorbance of eluates was measured at 600 nm in a spectrophotometer.

4. RESULTS AND DISCUSSION

It has been reported earlier [7] that E. coli ColV$^+$ strains auto-agglutinate in NB, whereas the isogenic strains lacking the ColV plasmid do not show this property. Other possible differences between the cell-surface characteristics of ColV$^+$ and ColV$^-$ E. coli strains were studied with the help of the-HIC technique.
The suspensions of *E. coli* strains were chromatographed on octyl-Sepharose in the presence of ammonium sulphate (0–1.0 M). The ColV + *E. coli* strains were found to be more strongly hydrophobic than their ColV− counterparts (Table 1). For example, the extent of adsorption to octyl-Sepharose of *E. coli* 1829 and its ColV + derivatives carrying ColV-K30 and ColV, I-K94 was 21.3%, 58.8% and 55.0% respectively, using 0.01 M phosphate buffer (pH 7.0), thus indicating the more hydrophobic nature of the ColV + strains of *E. coli* compared to the plasmidless parent. With the gradual increase in the concentration of ammonium sulphate in the phosphate buffer, there was an increase in the adsorption of the ColV− as well as ColV + strains of *E. coli* 1829, but with the extent of adsorption of the ColV + strains always being higher than that of the ColV− counterpart (Table 1). The ColV + derivatives of *E. coli* 1829 were almost completely retained by the octyl-Sepharose in the presence of 0.1 M ammonium sulphate, whereas ColV− *E. coli* 1829 required 5 times more ammonium sulphate to achieve the same results.

The association of hydrophobicity with the presence of ColV plasmids was confirmed by the transfer of ColV-K30 into another K12 strain (*E. coli* 1157) which lacked this plasmid initially (Table 1). Strain 1157 carrying ColV adhered to a much greater extent to octyl-Sepharose than the ColV− 1157 at all concentrations of ammonium sulphate tested. Similarly, transfer of ColV, I-94 to strain P678-54 led to increased hydrophobicity (Table 1), thus confirming the influence of ColV plasmids on the hydrophobicity of *E. coli*.

The results presented above suggest that the introduction of ColV-K30 or ColV, I-K94 into derivatives of *E. coli* K12 leads to increased hydrophobicity. There is, however, an alternative explanation. This is that some mutant organisms in the ColV− populations have altered surface properties which make them: (a) more hydrophobic; and (b) better recipients for ColV i.e. that the ColV + derivatives studied here have arisen by more efficient transfer of ColV into organisms which already have a more hydrophobic surface. Several findings rule out this possibility. Firstly, ColV-K30 and ColV, I-K94 are very efficiently transferred to strains 1829, 1157 and P678-54 (>10% of recipient strains become ColV + after 1 h incubation at 37°C with suitable donor ColV + strains) so that the proportion of mutant cells with hydrophobic surface properties would need to be unacceptably high. Secondly, if the increased surface hydrophobicity of ColV + strains were not dependent on the presence of the plasmid, then cured strains should still show increased surface hydrophobicity compared to the parental ColV− culture. This was not so: the hydrophobicity pattern of a cured 1829 strain was quite similar to that of the ColV− 1829 parent strain (Table 1). On re-introducing the ColV-K30 plasmid into the

### Table 1

The adherence to octyl-Sepharose of *Escherichia coli* strains carrying or lacking ColV

<table>
<thead>
<tr>
<th>Ammonium sulphate molarity</th>
<th>% Adherence</th>
<th>1829</th>
<th>1829</th>
<th>1829</th>
<th>Cured</th>
<th>1829</th>
<th>1829</th>
<th>1829</th>
<th>P678-54</th>
<th>P678-54</th>
<th>ColV, I-K94</th>
<th>1829</th>
<th>1829</th>
<th>1829</th>
<th>Flac</th>
<th>R124</th>
<th>R124</th>
<th>ColV, I-K94</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1829</td>
<td>1829</td>
<td>1829</td>
<td>1157</td>
<td>1157</td>
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</tr>
<tr>
<td>0.00</td>
<td>21.3</td>
<td>58.8</td>
<td>55.0</td>
<td>15.0</td>
<td>48.5</td>
<td>8.8</td>
<td>56.3</td>
<td>16.3</td>
<td>35.0</td>
<td>51.3</td>
<td>50.0</td>
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<tr>
<td>0.05</td>
<td>67.5</td>
<td>91.5</td>
<td>86.3</td>
<td>55.8</td>
<td>72.6</td>
<td>26.3</td>
<td>67.5</td>
<td>21.5</td>
<td>62.5</td>
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<td>67.5</td>
<td>96.3</td>
<td>93.0</td>
<td>62.5</td>
<td>86.3</td>
<td>33.8</td>
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<td>96.3</td>
<td>95.5</td>
<td>87.5</td>
<td>80.0</td>
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</table>

The percentage adherence was calculated from the absorbance of the eluate from the octyl-Sepharose columns compared to the absorbance of 0.1 ml of the original cell suspension in 4.0 ml of the phosphate buffer. The values are the average of two readings.
above-mentioned cured strain, its hydrophobicity pattern was found to be essentially the same as that of the original strain 1829 ColV-K30. Similarly, a derivative of strain 1829 made ColV, I-K94+ by transformation with plasmid DNA was similar in hydrophobicity to the ColV+ strain produced by conjugation (Table 1). These results further establish the role of ColV plasmids in enhancing the hydrophobicity of E. coli cells.

The above increases in hydrophobicity of ColV+ cells refer to growth at 37°C. Hydrophobicity of cells grown at 21°C was also tested. When the suspensions of ColV K30+ and ColV- 1829, grown at 21°C, were applied to the Sepharose columns, no significant difference in the percentage of adherence of the organisms, with and without ColV plasmid, was observed.

This finding (i.e. that growth at 21°C abolished the effect of ColV on bacterial hydrophobicity) suggested that ColV-specified sex pili might be responsible for the increased hydrophobicity since growth at low temperatures prevents synthesis of such pili.

Several other findings are in accord with sex pili being involved. Firstly, shearing of the 1829 ColV, I-K94+ strain (which is known to remove sex pili [10]) substantially reduced hydrophobicity. There was a slight effect on the Col- parent (this could be due to the removal of flagella and/or common pili) but the effect was substantially greater on the ColV, I-K94+ strain (Table 2). Incubation with cyanide causes retraction of F-specified sex pili [10] but did not affect the hydrophobicity of a ColV, I-K94+ strain (Table 2). It may be that ColV-specified sex pili are not retracted under these conditions but it is more likely that retraction does occur but leaves the tips of the pili exposed at the surface (as occurs for F-pili; [10]); it seems likely that it is the tip which would confer increased hydrophobicity (as the amino acids exposed on the sides of pili specified by F-like plasmids appear to be predominantly non-hydrophobic ones [12,13]).

Studies of the hydrophobicity of 1829 derivatives carrying other F-like plasmids support the view that ColV-specified sex pili may be responsible for the increased hydrophobicity. First, the presence of Flac, which specifies derepressed synthesis of sex pili, increased hydrophobicity (although the effect was less than for ColV) whereas presence of the repressed F-like plasmid, R124, did not (Table 1). Secondly, R124, which is a fin+ plasmid and stops synthesis of ColV-specified sex pili, completely reversed the effect of ColV, I-K94 on hydrophobicity (Table 1).

Finally, the conditions for synthesis at 37°C of the component(s) responsible for increased hydrophobicity are also in accord with this component being sex pilin or the pili formed from it. Assuming that the pro-pilin of ColV-specified sex pili resembles that specified by other F-like plasmids [12,13], then this component would contain a range of amino acids including leucine but would not contain histidine. Working on this assumption, the conditions for increase in hydrophobicity have been examined in strain 1157 ColV-K30 in supplemented minimal medium on a shift from 21°C to 37°C. With all required amino acids added (control), hydrophobicity increased substantially over the incubation period after the shift; in the absence of L-leucine (which essentially prevented growth, Table 3) there was only a small increase in hydrophobicity whereas the increase was essentially the same as in the control in the absence of

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**Table 2**

<table>
<thead>
<tr>
<th>E. coli strain</th>
<th>% Adherence to octyl-Sepharose</th>
<th>Blending experiment</th>
<th>NaCN experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Blended</td>
</tr>
<tr>
<td>1829</td>
<td>32.5</td>
<td>25.0</td>
<td>30.0</td>
</tr>
<tr>
<td>1829 ColV, I-K94</td>
<td>62.5</td>
<td>20.0</td>
<td>63.8</td>
</tr>
</tbody>
</table>

a Overnight grown cultures were blended (2 min) at 4°C, centrifuged and cells suspended in phosphate buffer (pH 7.0, 0.01 M). Washed cells were adjusted to the same A and 0.7 ml of the sample was applied to the O-Sepharose column. The values are the average of two readings.

b NaCN was added to exponentially grown cultures to a final concentration of 0.01 M. After 10 min the cells were centrifuged at 4°C, washed and suspended in phosphate buffer (pH 7.0, 0.01 M) and 0.1 ml of the sample was applied to O-Sepharose column.
Table 3
The adherence to octyl-Sepharose of *E. coli* 1157 ColV-K30 grown in minimal medium

<table>
<thead>
<tr>
<th>% Adherence of <em>E. coli</em> 1157 ColV-K30</th>
<th>Adherence after shift to 37°C in</th>
<th>Complete medium</th>
<th>Medium lacking leucine</th>
<th>Medium lacking histidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial adherence after growth at 21°C</td>
<td>15.0</td>
<td>60.0</td>
<td>30.0</td>
<td>50.0</td>
</tr>
</tbody>
</table>

Washed organisms were adjusted to the same A in the stated medium and incubated at 37°C for 5 h. Absorbance increased 130% for complete medium, 17% for medium minus leucine and 20% for medium minus histidine. Adherence to octyl-Sepharose was then measured as described in MATERIALS AND METHODS using columns equilibrated with 0.01 M phosphate buffer pH 7.0. Organisms grown at 37°C initially had % adherence of 55.0. The experiment was repeated twice with consistent results.

L-histidine although growth was abolished (Table 3). Accordingly, it appears that the component responsible for increased hydrophobicity of the ColV-K30+ derivative of strain 1157 can be formed minus L-histidine but not minus L-leucine.

All the above results are consistent with ColV-specified sex pili being important in increased hydrophobicity. However, it is possible that some other repressible transfer component(s) also plays a role.

Hydrophobic interactions have been reported to be important in the adhesion of bacteria to host cells [14]. Increase in hydrophobicity has been found to increase the association of *E. coli* and *Salmonella* spp. to human cells [15]. Therefore, it is suggested that one reason for the increased virulence of ColV+ strains of *E. coli* compared to ColV− ones might be that the increased surface hydrophobicity observed here allows stronger adherence to host cells.

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REFERENCES