Plasmid-Associated Cell Surface Charge and Hydrophobicity of
Yersinia enterocolitica

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Virulent strains of Yersinia enterocolitica and their plasmidless, avirulent derivatives were examined for their cell surface properties. Increased surface charge and hydrophobicity of Y. enterocolitica were found to be associated with the possession of a 40- to 48-megadalton plasmid. These surface properties were expressed, as were other plasmid-associated properties, at 37 but not at 22°C. The concentration of calcium in the growth medium had a moderate effect on the expression of the cell surface properties. These cell surface properties were greatly reduced among plasmid-bearing cells grown on tryptic soy agarose regardless of growth temperatures. These properties were also associated with the ability of Y. enterocolitica to colonize the gastrointestinal tract of mice.

Yersinia enterocolitica is a recognized enteric pathogen of humans (2, 29). Recent studies have demonstrated the involvement of a 40- to 48-megadalton plasmid in the virulence of this bacterium (7, 18, 28); a number of temperature-dependent properties associated with the virulence plasmid have been described (17, 29). The relationship between these properties and human virulence is not clear. Enterotoxin production does not appear to have an important role in the virulence of the organism (29).

The adherence of pathogenic bacteria to eucaryotic cells plays a central role in their ability to colonize mucosal epithelial surfaces (24), and the physicochemical properties of the bacterial surface are important in the host-parasite interactions. Recent studies have demonstrated that the hydrophobic character of bacteria plays a role in their interaction with mammalian cells (13, 26, 27). Thus, hydrophobic interaction is likely to provide the driving force for host-parasite interaction through displacement of water and formation of adhesive bonds. Electrostatic forces have also been shown to be involved in the attachment of bacteria to surfaces (19) and may be significant in mediating interaction between the bacteria and host cell surfaces. The combination of these physicochemical forces has been reported to increase the association of Escherichia coli and Salmonella spp. to the intestinal epithelium (13).

Little is known about the adherence properties of Y. enterocolitica. It has been suggested that the ability of the organism to attach to host cells is encoded in the chromosome (11, 18, 22). This is based on the observation that wild-type strains and their plasmidless derivatives are both invasive to HEP-2 or HeLa cells (18, 22). The present study reports the association of the virulence plasmid with colonization of the mouse gastrointestinal tract and with the altered physicochemical properties of the cell surface of Y. enterocolitica.

Four strains of Y. enterocolitica representing four serotypes (Table 1) were confirmed to be virulent by their ability to detach the monolayer of HEp-2 cells (18). In addition, the strains harbored the virulence plasmid which encoded for calcium growth dependency and autoagglutination. Derivatives of these strains cured of the virulence plasmid were obtained from large colonies (calcium growth independent) which emerged spontaneously in cultures growing at 37°C in magnesium chloride-oxalate agar (8). These plasmidless strains were lacking autoagglutination and lost their ability to detach the HEP-2 cell monolayer. Groups of three Swiss albino mice weighing 15 to 20 g were orally infected with Y. enterocolitica strains (7, 12, 25); the presence of diarrhea and the condition of the stools were observed. Stools were analyzed for Y. enterocolitica by using McConkey agar plates and the API 20E system (Analytab Products, Inc., Plainview, N.Y.). Among the plasmid-bearing strains, only the serotype O:8 strain was lethal to mice; death occurred within 2 days after oral challenge. The rest of the virulent strains failed to evoke obvious symptoms of illness. However, the mice continued to shed the bacteria in their stools throughout the course of the experiment (14 days). In contrast, the plasmidless strains were no longer detectable in the mouse stools 3 days after oral challenge. Similarly, Pai and DeStephano (16) demonstrated that virulent strains of Y. enterocolitica established foci of infection on the Peyer's patches of mice, whereas the avirulent counterparts were quickly eliminated after oral challenge. These observations indicate that the colonization of the mouse intestine by Y. enterocolitica is plasmid mediated. This conclusion is at variance with the generally held notion that the attachment function in Y. enterocolitica is encoded in the chromosome on the basis of the capability of both plasmid-bearing cells and their plasmidless derivatives to penetrate HEp-2 or HeLa cells (18, 22). Yet, HeLa cell penetration by inanimate particles such as thorium dioxide has been documented (1).

We also observed that the virulence plasmid of Y. enterocolitica encoded for a highly anionic and hydrophobic cell surface. In addition, the expression of these cell surface properties was growth medium and temperature dependent. The hydrophobicity of the cell surface of Y. enterocolitica
TABLE 1. Relationship of the virulence plasmid, virulence, and other phenotypic properties of *Y. enterocolitica* strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serotype</th>
<th>Source</th>
<th>Plasmid&lt;sup&gt;a&lt;/sup&gt; (40- to 48-Md)</th>
<th>CAD&lt;sup&gt;b&lt;/sup&gt;</th>
<th>AA&lt;sup&gt;c&lt;/sup&gt;</th>
<th>HEP-2 cell detachment&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Fecal shedding of bacteria&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 2635</td>
<td>O:8</td>
<td>J. C. Feeley</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Lethal</td>
</tr>
<tr>
<td>TAMU 75</td>
<td>O:8</td>
<td>D. L. Zink</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MCH 56A2</td>
<td>O:3</td>
<td>C. H. Pai</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MCH 56A2-C</td>
<td>O:3</td>
<td>This study</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PG1-286</td>
<td>O:5, O:27</td>
<td>C. Vanderzant</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PG1-286-C</td>
<td>O:5, O:27</td>
<td>This study</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>78-513</td>
<td>O:1, O:2, O:3</td>
<td>W. H. Lee</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>78-513-C</td>
<td>O:1, O:2, O:3</td>
<td>This study</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> The method of Kado and Liu (10) were used for the determinations of plasmid DNA. Md, Megadalton.

<sup>b</sup> CAD. Calcium growth dependency (15) was determined by using tryptic soy agar as the basal medium.

<sup>c</sup> AA. Autoagglutination. The autoagglutination test was done by the method of Laird and Cavanaugh (12).

<sup>d</sup> HEP-2 cell detachment was performed as described by Portnoy et al. (18).

<sup>e</sup> Mice fecal excretion of *Y. enterocolitica* for 14 days after oral challenge.

was first indicated by the adherence of the cell to the polystyrene surface (20). Plasmid-bearing cells from colonies grown at 37°C on brain heart infusion agar plates adhered tenaciously to the plastic surface. On the other hand, the plasmidless cells were easily displaced from the surface by washing. Plasmid-bearing cells grown on tryptic soy agar or at 22°C were easily dislodged from the polystyrene surface. Cell surface hydrophobicity was also measured by using a quantitative assay described by Rosenberg et al. (21). When xylene or some other water-immiscible solvent was mixed with an aqueous suspension of cells, the cells exhibited an affinity for one of the two phases upon partitioning. Plasmid-bearing cells grown in tissue culture medium at 37°C tended to bind to the xylene phase (Fig. 1). When the results were quantified by measuring the remaining opacity of the aqueous phase, we saw that the extent of the adherence of the cells to the xylene layer was a function of the xylene concentration. When grown at 22°C, the plasmid-bearing cells had less affinity to xylene. Strains cured of their plasmid, regardless of the growth temperature, had little tendency to partition into the nonaqueous phase.

The tendency of xylene to lyse the cells caused us to use another method for quantifying the hydrophobic property of the cells. Recent studies suggested that hydrophobic interaction was responsible for the binding of ribosomes to nitrocellulose filter (NCF) (3, 5). We explored the utility of NCF in discriminating among bacterial strains with respect to the hydrophobicity of their surfaces. Washed cells suspended in a final volume of 3 ml in saline solution (0.9% NaCl, pH 7.2) were added to culture tubes (12 by 75 mm). The optical density at 600 nm (1-cm pathlength) of the cell suspension was adjusted to ca. 1.0. This suspension was passed through (dropwise) a 13-mm NCF (type SC, 8.0-μm pore size; Millipore Corp., Bedford, Mass.) at a rate of 1 drop per s. A 1-ml amount of the filtrate was transferred to a cuvette, and its optical density was determined. Control samples consisted of cell suspensions not passed through the filter.

Plasmid-bearing cells grown on brain heart infusion plus 1.2% agarose (a calcium-deficient medium) at 37°C had strong affinity to NCF (Fig. 2). This was greatly diminished when the cells were grown at 22°C or on tryptic soy agarose. There was a moderate reduction in the cells affinity to NCF when grown in the presence of added calcium (2.5 mM) or on brain heart infusion agar. Plasmidless cells, on the other hand, had little affinity for the filter regardless of growth conditions.

Cell surface charge of *Y. enterocolitica* was examined by using a modification of the hydroxyapatite (HA) adherence

![Figure 1](http://iai.asm.org/)

**FIG. 1.** Partitioning of *Y. enterocolitica* strain MCH 56A2 (p<sup>+</sup>) and its plasmidless derivative (p<sup>-</sup>) grown for 24 h at 37 or 22°C.

![Figure 2](http://iai.asm.org/)

**FIG. 2.** NCF adsorption of *Y. enterocolitica* strain MCH 56A2 (■) and its cured derivative (□) cultivated under various growth conditions. BHIO, Brain heart infusion plus 1.2% agarose; TSO, tryptic soy agarose.
assay of Ericson et al. (4). Adherence of the cells to HA was indicated by the reduction in opacity of the cell suspension after the addition and sedimentation of the HA particles. The procedure involved the suspension of bacteria in saline or in saline containing either 10 mM CaCl₂ or 10 mM phosphate buffer. Each of bacterial suspensions was added to a 16-by-150-mm test tube containing 100 mg of HA (Calbiochem, La Jolla, Calif.) and mixed for 1 min. The HA particles were allowed to settle out of suspension at 22°C for 30 min. A 1-ml amount of the remaining cell suspension was transferred to a cuvette, and its optical density was determined. The plasmid-bearing cells were much more adherent to HA than the plasmidless cells (Fig. 3). The addition of calcium increased the adherence of the cells to HA, whereas the addition of phosphate ions had the opposite effect. The results are consistent with the concept that binding of the cells to HA involves an electrostatic interaction taking place between the negatively charged cell surface and the calcium site on the crystal surface (19). The addition of calcium promotes the bridging between the negatively charged cell surface and the phosphate site on the HA surface. This model is similar to the one described by Rölla et al. (19) involving the interaction between Streptococcus mutans and HA.

The same growth conditions which influence the expression of cell surface hydrophobicity affect the expression of cell surface charge as shown in Fig. 4. The components in the tryptic soy agarose affecting the expression of the cell surface properties of Y. enterocolitica remains to be determined.

Difficulties were initially encountered when broth cultures in the study. These were due to the high rate of curing of the plasmid (28) and the rapid rate of growth of plasmidless mutants at 37°C (7). The problem was circumvented by using semisolid media, with which grown colonies consisting mostly of plasmid-bearing cells can be differentiated from colonies of plasmidless cells. In addition, the hydrophobicity of the cells was monitored by using the latex agglutination test (R. V. Lachica and D. L. Zink, in press).

The physicochemical properties on the surface of virulent strains of Y. enterocolitica may play an important role in conferring or modulating adherence properties of the bacterium to the epithelial tissue during pathogenesis. A combination of these physicochemical properties has been associated with increased adherence of E. coli and Salmonella spp. to the intestinal epithelium (13). Similar observations have been reported with Vibrio cholerae (6, 9). It is envisaged that an increased charge on the cell surface of the plasmid-bearing cells provides binding sites to which divalent cations, such as calcium, can adsorb to bridge the bacterium to the mucosal surface of the gut. This ionic interaction is then stabilized by hydrophobic groups adjacent to the sites of bond formation. Similar mechanisms have been suggested on the adherence of oral streptococci to tooth surfaces (14, 19). It remains to be determined whether a more specific adherence mechanism (surface-ligand binding) (24) may also be operative in the attachment of Y. enterocolitica to the mucosal surface.

As with other plasmid-associated properties of Y. enterocolitica (17, 29), the altered physicochemical properties of the cell are temperature dependent. However, unlike the V antigen (17) and the plasmid-associated outer membrane proteins (18), these cell surface properties are only moderately repressed by calcium. These observations are consistent with the possibility that after ingestion, the bacterial cells are induced to synthesize a plasmid-associated surface structure to mediate attachment to the intestinal mucosa. Subsequently, after host penetration, the intracellular condition of low calcium content (23) signals the biosynthesis of plasmid-associated outer membrane proteins and the V and W antigens (17, 18).

We have identified a surface structure which may mediate the attachment function in Y. enterocolitica (R. V. Lachica and D. L. Zink, manuscript in preparation). Our preliminary results indicate the formation on the surface of the cell of a plasmid-associated anionic fibrillar structure which differs from the fimbrae described by Old and Robertson (15).

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LITERATURE CITED
