Virulence plasmid-associated sensitivity to acid in *Escherichia coli* and its possible significance in human infections

G. E. COOPER and R. J. ROWBURY

Department of Botany and Microbiology, University College London, Gower Street, London WC1E 6BT

Summary. Several strains of *Escherichia coli* were markedly sensitised to killing at pH 2·5 or 3·5 when the ColV,I-K94 virulence plasmid was introduced into them. For strain 1829, the effect on acid sensitivity was due to the presence of plasmid in the previously resistant strain rather than to its introduction into an acid-sensitive variant already in the population. Acid sensitivity was also conferred by the ColV-K30 and ColB-K98 plasmids and the resistance plasmid R124-F2; other plasmids tested had no marked effect. Studies of ColV⁺ strains carrying mutant plasmids indicated that it was the presence of ColV-encoded transfer components that made ColV,I-K94⁺ strains acid-sensitive. Organisms in the exponential phase of growth were more sensitive to acid than were those from stationary phase cultures and this difference was more marked for ColV,I-K94⁺ strains than for Col⁻ ones. Moreover, ColV⁺ strains exposed to conditions of low pH for short periods subsequently grew less well than the Col⁻ parent and appeared to be sensitised by the damage to the effects of H₂O₂. These results indicate that some ColV⁺ strains may be more sensitive to gastric acid and to phagocytic acidity than are Col⁻ strains. ColV,I-K94⁺ strains grew as well as Col⁻ ones in broth or urine at pH 4·5–6·0 which suggests that the presence of the plasmid would not be detrimental to bacterial growth in the urinary tract. The presence of transfer components in the outer membrane of ColV,I-K94⁺ bacteria may destabilise the lipopolysaccharide layer allowing increased penetration of hydrogen ions.

Introduction

Plasmids of the ColV group are large elements that confer F-like transfer properties on enterobacteria that harbour them. Such transfer is derepressed for many of these elements (Hardy, 1975) although transfer efficiency can be greatly reduced by the presence of *fin*⁺ plasmids. ColV plasmids also permit the organisms to synthesise the colicin V toxin, its immunity component (and, frequently, colicin Ia and its immunity component) and the VmpA outer membrane protein (Moores and Rowbury, 1982; Rowbury *et al.*, 1985).

The virulence-enhancing effect of ColV plasmids on enterobacteria probably results from several different properties. First, ColV plasmids increase the serum resistance of *Escherichia coli* strains, allowing them to survive the effects of complement (Williams Smith, 1974; Binns *et al.*, 1979 and 1982; Taylor, 1983). Second, some ColV plasmids encode the aerobactin iron chelation system (Williams,

1979) which permits growth in iron-depleted serum and confers greater resistance to phagocytic killing (because the organisms are able to maintain their catalase activity). Third, the presence of a ColV plasmid enables the organism to attach to host cells (Clancy and Savage, 1981), perhaps because of increased hydrophobicity of the surface of ColV⁺ organisms (Tewari *et al.*, 1985). Furthermore, the autoagglutination of ColV⁺ strains may enhance survival in the animal host (Rowbury *et al.*, 1985).

Strains harbouring ColV plasmids have been isolated from patients with serious intestinal infections (Fredericq and Joiris, 1950). Moreover, ColV plasmids can enhance invasiveness (Williams Smith, 1974; Williams Smith and Huggins, 1976) and ColV⁺ organisms are often responsible for systemic infections in livestock and urinary tract infections in man.

To reach and multiply in the intestine, ingested organisms must survive exposure to gastric acid (*c.* pH 2). An organism which subsequently invades the tissues and bloodstream from the intestine will then be subjected to attack by phagocytes, within the

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Please address all correspondence to Dr R. J. Rowbury.

phagolysosomes of which, *pH* values of 3.5–4.5 or less can be achieved (Jensen and Bainton, 1973; Mims, 1982; Rous, 1925). Few enterobacteria will multiply at *pH* 4.5 but the ability of phagocytosed organisms to survive this acidity might aid pathogenicity if lysis of the phagocyte allowed release of viable organisms. Potential pathogens also have to resist acid in the vagina, where *pH* values of *c.* 5.0–5.5 are produced by commensal lactobacilli, and in urine, where similar *pH* values are found. The growth rates of most pathogens would be markedly reduced at such *pH* values. Changes in acid sensitivity associated with introduction of ColV plasmids may, therefore, be relevant to pathogenicity of ColV⁺ strains and we have examined this possibility.

Materials and methods

Bacterial strains

All strains were derivatives of *E. coli* K12. The characteristics of strains 1829, P678–54 and 1157 have been described previously (Tewari *et al.*, 1985). The ColV,I-K94⁺ strains and derivatives of strain 1829 carrying other plasmids were prepared by plasmid transfer from *E. coli* J53 (Moore and Rowbury, 1982; Tewari *et al.*, 1985); in one case, ColV,I-K94 was introduced into *E. coli* strain 1829 by transformation (Tewari *et al.*, 1985). *E. coli* strain 1829 ColV,I-K94 was cured of plasmid carriage by growth at elevated temperature (Rowbury *et al.*, 1985).

Growth media and culture conditions

Nutrient Broth (Oxoid No. 2, 25 g/L), solidified as required with 2% w/v Bacto Agar (Difco), was used; the *pH* of broth media was appropriately adjusted with lactic acid.

For growth tests in urine, pooled freshly voided samples from men were filtered through 1.2- μ m pore size membrane filters and adjusted to the appropriate *pH* with lactic acid or NaOH.

Unless otherwise stated, organisms were grown to exponential phase with shaking.

Acid treatment

For tests of viability at *pH* 2.5 or 3.5, organisms were diluted into broth acidified with HCl and incubated for the required period at 37°C. Treated organisms were then diluted with 0.05M Tris-HCl buffer (*pH* 7.5) and samples plated on nutrient-agar plates and incubated at 37°C.

Growth of organisms exposed to *pH* 2.5 or 3.5 in broth was also investigated. After the appropriate length of exposure, organisms were diluted into fresh broth, the *pH* was adjusted to 7.35–7.4 and incubation was continued with shaking at 37°C. Samples were taken at intervals for

optical density measurement at 520 nm. Some of the acid-treated samples were subsequently exposed to hydrogen peroxide (2.5 mM); after the required exposure to H₂O₂ organisms were harvested, resuspended in fresh broth and re-incubated as above.

Results

Sensitivity of *E. coli* strains to *pH* 2.5

The presence of the ColV,I-K94 plasmid greatly increased the sensitivity of *E. coli* strain 1829 to killing at *pH* 2.5 (fig. 1). Similar effects were seen with *E. coli* strains 1157 and P678–54 (table I). Another ColV plasmid (ColV-K30) also markedly increased the acid sensitivity of *E. coli* strain 1829 whereas a cured derivative of strain 1829 ColV,I-K94 was as resistant as strain 1829 itself (table I).

Acid sensitivity of organisms harvested from exponential-phase cultures was greater than that of organisms harvested from stationary-phase cultures for both ColV,I-K94⁺ organisms and for their Col[−] parent, but the difference between stationary and exponential cells was greater for the ColV,I-K94⁺ strains (table II).

Fig. 1. The sensitivity of *E. coli* strains 1829 and 1829 ColV,I-K94 to *pH* 2.5. ○ = strain 1829; ● = strain 1829 ColV,I-K94.

Table I. Effect of ColV plasmids on acid sensitivity

<i>E. coli</i> strain	Percentage viability at pH 2.5*
1829	100
1829 ColV,I-K94	1
1829 ColV-K30	0
1829 cured	115
1829 ColV,I-K94 transformed	3
1157	100
1157 ColV,I-K94	3
P678-54	100
P678-54 ColV,I-K94	3

* Viability relative to that of the plasmid-free parent (100%) after incubation for 10 min in broth at pH 2.5.

Table II. Acid sensitivity of organisms from exponential and stationary phases of growth

<i>E. coli</i> strain	Percentage survival after exposure to pH 2.5 for 10 min of cells u	
	Stationary-phase	Exponential-phase
1829	27	3.3
1829 ColV,I-K94	23	0.2
1157	10	0.2
1157 ColV,I-K94	5	0.002
P678-54	42	34
P678-54 ColV,I-K94	25	2.2

Effects of other plasmids on acid sensitivity

A derivative of *E. coli* strain 1829 carrying a mutant form of plasmid ColV,I-K94—ColV-M50(1)—which encodes normal colicin components but no transfer components, exhibited acid sensitivity very similar to that of *E. coli* strain 1829 itself, i.e., loss of transfer components led to resistance (table III). In contrast, the presence of plasmid ColV-M40(5), which confers normal transfer properties but no colicin components, made *E. coli* strain 1829 almost as acid-sensitive as strain 1829 ColV,I-K94 (table III).

Several other plasmids were tested for their effects on acid sensitivity when introduced into *E. coli* strain 1829. Plasmid R124-F2, which confers derepressed transfer properties, had a marked sensitising effect whereas *Flac* had little effect (table III). The resistance plasmid R124 (the parent of plasmid R124-F2) alone had little effect on the acid sensitivity of *E. coli* strain 1829; when present with ColV,I-K94, it abolished the effect of the latter

Table III. Plasmid components and acid sensitivity

<i>E. coli</i> strain	Colicin/immunity components	Transfer components	Percentage viability at pH 2.5*
1829	none	none	100
1829 ColV,I-K94	V and Ia	derepressed	3
1829 ColV-M40(5)	none	derepressed	13
1829 ColV-M50(1)	V and Ia	none	67
1829	none	none	100
1829 ColV,I-K94	V and Ia	derepressed	3
1829 <i>Flac</i>	none	derepressed	70
1829 R124-F2	none	derepressed	27

* Relative to *E. coli* strain 1829 (100%).

Table IV. Repression of transfer properties and acid sensitivity

<i>E. coli</i> strain	Colicin/immunity components	Transfer components	Percentage viability at pH 2.5*
1829	none	none	100
1829 ColV,I-K94	V and Ia	derepressed	11
1829 R124	none	repressed	89
1829 ColV,I-K94 R124	V and Ia	repressed	89
1829	none	none	100
1829 ColV,I-K94	V and Ia	derepressed	2
1829 ColB-K98	B and M	repressed	13

* Relative to *E. coli* strain 1829 (100%).

(table IV). The plasmid ColB-K98 markedly increased the acid sensitivity of *E. coli* strain 1829 (table IV).

Effect of ColV,I-K94 at pH 3.5

For all three *E. coli* strains tested (strains 1829, 1157 and P678-54), organisms carrying plasmid ColV,I-K94 were markedly more sensitive to killing at pH 3.5 than were the Col⁻ parents. The percentages of *E. coli* strain 1829 and strain 1829 ColV,I-K94 cells surviving at pH 3.5 during incubation for 60 min at 37°C are shown in fig. 2.

Growth inhibition after exposure to acid

Growth rates of organisms were reduced after exposure to either pH 2.5 or pH 3.5 for short periods and the effects were much more marked for ColV⁺ strains than for Col⁻ strains. Fig. 3 shows that, after exposure to pH 3.5 for 12.5 or 16 min at 37°C,

Fig. 2. The sensitivity of *E. coli* strains 1829 and 1829 ColV,I-K94 to pH 3.5. ○ = strain 1829; ● = strain 1829 ColV,I-K94.

organisms of *E. coli* strain 1829 ColV, I-K94 grew less well than those of strain 1829 itself. Similar results were obtained with *E. coli* strain 1157 and P678-54 and their ColV,I-K94⁺ derivatives.

Organisms surviving damage by acid appeared to be sensitised to growth inhibition by H₂O₂ (table V).

Growth and multiplication at pH 4.0–6.0

There was no significant difference between *E. coli* strain 1829 and strain 1829 ColV,I-K94, with respect to growth in broth acidified with lactic acid.

Fig. 3. Growth inhibition after exposure of *E. coli* strains 1829 and 1829 ColV,I-K94 to pH 3.5. ○ = strain 1829 untreated; ● = strain 1829 after 12.5 min at pH 3.5; ○ = strain 1829 after 16 min at pH 3.5; ▽ = strain 1829 ColV,I-K94 untreated; ▼ = strain 1829 ColV,I-K94 after 12.5 min at pH 3.5; X = 1829 ColV,I-K94 after 16 min at pH 3.5. Organisms grown throughout in broth at 37°C, shaken.

Table V. Sensitisation of *E. coli* strain 1829 ColV,I-K94 to H₂O₂ by acid treatment

Treatment	Growth at 90 min*
None	100
Acid† 5 min	79
H ₂ O ₂ 5 min	106
H ₂ O ₂ 10 min	115
Acid 5 min, H ₂ O ₂ 5 min	25
Acid 5 min, H ₂ O ₂ 10 min	24

* Percentage of untreated control.

† Acid treatment, pH 3.5.

Neither strain grew or changed the pH during incubation for 3.5 h at 37°C in broth of initial pH ≤ 4.3. Both strains grew slightly in broth initially at pH 4.4 or 4.5 and substantial growth of both strains occurred at pH ≥ 4.6.

Growth in urine was poor. With an inoculum of $c. 3 \times 10^{10}$ organisms/L, neither *E. coli* strain 1829 nor strain 1829 ColV,I-K94 grew appreciably during incubation for 24 h in urine of which the initial pH was 4.5. Cell mass (dry weight/ml) approximately doubled for both strains when the initial pH was 5.0. Better growth was achieved at pH 5.5 and 6.5 but there appeared to be little difference between the parent *E. coli* and its ColV,I-K94⁺ derivative.

Discussion

ColV plasmids confer or enhance several bacterial properties that may be related to virulence. Amongst these properties, the production of iron-chelating components does not depend on derepressed transfer properties and the same may be true for increased resistance to complement (Nilius and Savage, 1984). In contrast, adhesion properties (Clancy and Savage, 1981), increased surface hydrophobicity (Tewari *et al.*, 1985) and autoagglutination, which may impede phagocytosis and killing by inhibitory agents (Rowbury *et al.*, 1985), depend on derepressed transfer properties. Accordingly, maintenance of these potential virulence properties depends on the plasmid transfer properties being derepressed. The results described here indicate that as well as conferring beneficial effects, the ColV plasmids expose the host bacterium to extreme acid sensitivity. This sensitivity is due to the presence of the plasmid and not to its introduction into an acid-sensitive variant because curing the plasmid reduced acid-sensitivity to that of the parental Col⁻ strain (table I). The acid sensitivity appears to depend on the presence of transfer components because factors that lead to loss or repression of transfer properties, reduced or abolished the acid sensitivity conferred by ColV plasmids (tables III and IV). The effects of transfer components is specific for individual plasmids because R124-F2 conferred marked acid sensitivity whereas *Flac* had

no effect. The sensitising effect conferred by ColV-K98 must depend on some other component because transfer properties of this plasmid are repressed. The transfer components may confer acid sensitivity by allowing hydrogen ions to cross the outer membrane more readily. The tight packing of the lipopolysaccharide (LPS) molecules maintains the impenetrability of the outer membrane to many inhibitory agents and insertion of transfer components into the outer membrane may weaken LPS-LPS bonds, as has been suggested for compounds with strongly cationic regions (Hancock, 1984). This could then allow increased permeation and acid killing. Alternatively, certain transfer components (e.g., pili) might act as pores to allow increased acid permeation across the outer membrane.

Increased acid sensitivity encoded by the ColV plasmids tested would reduce survival of ColV⁺ organisms on exposure to gastric acid to the acid environment in phagolysosomes. ColV⁺ survivors might also be sensitised to the effects of other inhibitory agents, as we observed here with H₂O₂ (table V).

The ColV plasmids had no marked effect on growth in broth or urine at the acid pH values (5.0–6.0) commonly found in urine. Accordingly, the virulence characteristics aiding survival in this situation, e.g., adhesion properties, are not counterbalanced by detrimental effects in relation to pH.

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