BACTERIAL PATHOGENICITY

Effect of pH, temperature and surface contact on the elaboration of fimbriae and flagella by Salmonella serotype Enteritidis

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Survival of enteric pathogens exposed to various environmental stresses depends upon a number of protective responses, some of which are associated with induction of virulence determinants. Flagella and fimbriae are putative virulence determinants of Salmonella spp. and ELISAs specific for the detection of flagella and SEF21, SEF14 and SEF17 fimbriae were used to assess the effect of temperature and pH upon their elaboration by isolates of Salmonella serotype Enteritidis in planktonic growth and on the surface of two-dimensional gradient agar plates. For three phage type 4 isolates of Enteritidis of comparative clinical provenance, similar phenotypes for the elaboration of these surface antigens were observed. SEF14 fimbriae were elaborated in planktonic growth at 37°C, but not 20°C, at pH 4.77 and above but not at pH 4.04; whereas on agar gradient plates SEF14 fimbriae were elaborated poorly but with best yields at pH 4.04. SEF17 fimbriae were elaborated in planktonic growth at 20°C, but not at 37°C, at pH 6.18 and above but not at pH 5.09 or below; whereas on agar gradient plates SEF17 fimbriae were elaborated well even at pH 4.65. SEF21 fimbriae were expressed very poorly under all conditions tested. Planktonic growth at 37°C induced least flagella whereas growth at 20°C, and particularly surface growth at lower pH values, induced a ‘hyper-flagellate’ phenotype. Single colonies allowed to form on gradient agar plates were shown to generate different colonial morphologies which were dependent on initial pH. These results demonstrate that the physicochemical environment is an important determinant of bacterial response, especially the induction of putative virulence factors.

Introduction

Salmonella serotype Enteritidis phage type (PT) 4 remains prevalent as a major cause of foodborne infections in the UK and Europe with poultry and poultry products, including eggs and processed foods containing egg, cited as common sources [1, 2]. Enteritidis is invasive in poultry, with contamination of muscle and reproductive tissues documented [3, 4], and persists in large numbers in the gut [5, 6], which contributes to surface contamination of eggs upon passage through the cloaca and to surface contamination of meat during evisceration [7, 8]. The fact that human infections arise indicates that Enteritidis is environmentally tolerant and survives within these various food environments. It has been established that many stress-related responses and virulence determinants are co-regulated with, for example, RpoS, the so-called stationary phase RNA polymerase sigma factor [9, 10], associated with responses to nutrient depletion, acidic pH and elevated temperatures [11] and the regulation of the virulence plasmid spv genes in Salmonella [12–14]. The question arises as to whether other putative virulence factors are up-regulated during stress, or more specifically, during survival or growth within foods.

The flagella and fimbriae of S. serotype Typhimurium are regarded as virulence determinants which play a role in initial colonisation of gut epithelia [15] and invasion [16–18]. Whilst it is well known that type 1 fimbriae mediate mannose-sensitive haemagglutination (MSHA) and play a role in gut epithelium adherence [19–21], other fimbriae, such as the long polar fimbriae (LPF) and the plasmid-encoded fimbriae (PEF), enable adherence to M cells in the Peyer's

Received 26 Feb. 1998; revised version accepted 13 July 1998.
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patches and cause fluid accumulation in ligated gut loops, respectively, in a mouse model [22, 23].

Enteritidis elaborates SEF21 fimbriae, which mediate MSHA [24, 25], and SEF17 [26, 27], an analogue of the ‘curli’ fimbriae of *Escherichia coli* [28] which mediate binding of fibronecin [29], plasminogen [30] and human contact factors [31]. Similar properties have been demonstrated for SEF17 [32], which may support the hypothesis that this kind of fimbria may be important in the pathogenesis of salmonella infection [33]. Enteritidis elaborates SEF14 fimbriae that are unique to a subset of group D *Salmonella* [34–37], although their role in pathogenesis remains equivocal [38]. Enteritidis elaborates PEF fimbriae, which are expressed during infection in chickens [39], but their role in pathogenesis is in doubt because plasmid-free variants remain fully virulent in chickens [40]. Enteritidis has the genetic potential to elaborate LPF, although the fimbrial structure has not yet been formally identified [22, 23, 41].

The role of Enteritidis fimbriae in pathogenesis in chickens has been studied in this laboratory. Of interest is the potential up-regulation of these putative virulence determinants during survival and growth, particularly within foods and other environments. The availability of monoclonal antibodies (MAbs) specific to flagella and SEF21, SEF14 and SEF17 fimbriae [25, 37, 42; and Sojka et al. unpublished observations] has enabled studies of their elaboration under different environmental conditions. The initial findings of the studies are reported here.

Materials and methods

Organisms and inocula

*Salmonella* Enteritidis strains S1400 and LA5, wild-type strains isolated from natural chicken infections, have been characterised in this laboratory [42, 43] and are considered similar to strain E [44, 45]. Strains E and I, kindly provided by Professor T. J. Humphrey (PHLS Food Microbiology Research Unit, Exeter), represent a virulent, environmentally tolerant strain and an avirulent, environmentally sensitive strain, respectively [44, 45]. In consultation with Professors T. J. Humphrey and G. S. A. B. Stewart, we have confirmed that strain I possesses a deletion of c. 400 bp in the *rpm* locus (these findings will be reported elsewhere). Enteritidis strain 27655R [46], originally isolated as an ‘atypical’ rough strain, and a naturally occurring derivative strain 27655S [46] were kindly provided by Professor T. Wadstrom (Lund University, Sweden). All cultures were maintained on Dorset’s egg slopes at 4°C. All inocula were prepared by direct inoculation of Trypticase Soy Broth (TSB; Becton Dickinson, Cockeysville, MD, USA) from stock cultures, followed by incubation at 25°C for 24 h and transfer of 100 μl to fresh TSB with incubation for a further 24 h at 20°C.

Broth culture medium

Ten-ml volumes of colonisation factor antigen (CFA) broth comprising (w/v) casamino acids 1.0%, yeast extract 0.15%, MgSO₄ 0.005% and MnCl₂ 0.0005% were adjusted to the appropriate pH with 1 M HCl or 1 M NaOH, filter-sterilised and inoculated with 100 μl of culture. These were incubated at 37°C or 20°C for 24 or 48 h.

Construction of neutral-pH4 gradient plates

Gradient plates were prepared according to the methods of Thomas et al. [47] and were constructed as follows: water agar, which comprised agar (Difco) 7.5 g suspended in 242 ml of distilled water was sterilised by autoclaving (121°C for 15 min) and cooled to 50°C. Double-strength CFA broth (250 ml), previously sterilised and held at 50°C, was mixed with the water agar and 8 ml of sterile 1 M HCl were added to obtain a pH value of 4.0. Molten culture medium in 40-ml volumes were poured into 12 cm² petri dishes (Bibby Sterilin, Stone) raised at one end by 6 mm and allowed to set. Molten CFA agar was prepared as above, but with sterile 1 M NaOH added to obtain a pH value of 7.0 and 40-ml volumes were poured over the acid gradient layer but with the plates level. When set, the plates were left for 24 h at 20°C to allow the pH gradients to form, and were then flooded with 10 ml of culture medium diluted to 10⁶ cfu/ml in quarter-strength Ringer’s solution (Oxoid) to generate confluent growth upon incubation. The pH gradient of an uninoculated plate was determined before inoculation with a double-junction, flat-ended pH electrode (BDH, Leicester) connected to a Corning 240 pH meter (BDH).

ELISA assays for surface antigens

The ELISA assays were based on those described previously [25, 37, 42]. Briefly, a cork borer (10-mm diameter), sterilised by dipping in ethanol 70% w/v and flaming, was used to extract two cores of medium. Bacteria adhering to the surface of the bores were loosen by vortex mixing in 2 ml of carbonate buffer, pH 9.6, and the resulting homogenate was adjusted to an optical density at 540 nm (OD₅₄₀) of 1.20. Broth cultures were pelleted by centrifugation at 4800 g and resuspended in carbonate buffer to an OD₅₄₀ of 1.2. Samples of culture homogenate (100 μl) were used to coat microtiratation plates (Polyisorb, Nunc, Glasgow). Microtitration plates were incubated at 37°C for 24 h then washed twice with 0.1 M phosphate-buffered saline (pH 7.2) containing Tween 20 0.05% v/v. Each well was blocked with 200 μl of skimmed milk solution 3% w/v, then treated as appropriate, in separate experiments, with mouse-derived MAbs specific for SEF14, SEF17, SEF21 fimbriae and flagella. Bound MAb was detected by goat anti-mouse horse-radish peroxidase-conjugated antibody (Sigma) developed with tetramethylbenzidine substrate (TMB; Cambridge Veterinary Sciences, Cambridge) at 20°C.
for 5 min and reactions were stopped with 100 µl of sulphuric acid 10% v/v. OD₄₅₀ measurements were noted. Triplicate samples were processed for each variable tested and control wells, which contained no primary antibody, were used as blanks.

Results

Elaboration of fimbriae and flagella

Six Enteritidis strains were tested for elaboration of flagella, type 1 (SEF21), SEF14 and SEF17 fimbriae in the growth conditions described above; the results are summarised in Table 1.

At 37°C, strains LA5, S1400, E, I and 27655S showed consistent detectable changes in the elaboration of flagella and SEF14 fimbriae that were related to the initial pH and physical nature of the growth medium. More flagella were elaborated on agar than in broth culture, except at the lowest pH value tested (pH 4.04), where elaboration of flagella in planktonic and on surface culture appeared equivalent. Conversely, elaboration of SEF14 was significantly greater in broth than agar culture except at pH 4.04, where SEF14 elaboration by planktonic culture was negligible (OD₄₅₀ < 0.10) but was at its greatest for the agar surface culture. It was of interest that, although the trends for all five cultures were very similar, the yield of SEF14 fimbriae by surface agar cultures of strains I and 25667S was distinctly greater than for strains LA5, S1400 and E, recognised as virulent strains. For these five strains, elaboration of SEF21 fimbriae was low (OD₄₅₀ c. 0.10) for all culture conditions. Elaboration of SEF17 was not detectable at 37°C. Strain 27655R has been described previously as producing an unusual, rough colonial morphology [27, 46], shown to be associated with expression of SEF17 fimbriae, possibly constitutively [42]. These data confirm that SEF17 fimbriae were elaborated at the non-permissive temperature (37°C). Again, SEF21 fimbriae were elaborated in greater amounts than for the other five test strains and at the lowest pH, irrespective of planktonic or surface growth.

At 20°C, strains LA5, S1400 and E showed similar phenotypes. Elaboration of flagella was similar for both planktonic and surface agar growth, although reduced on surface agar growth at the lower pH and especially for strain E; this phenomenon was also noted for strain I. Elaboration of flagella by strains 27655S and 27655R was similar, not surprisingly in that strain 27655S was derived from strain 27655R, and notable in that yields were greater for surface-grown than broth-grown culture and greater than for the same or any other strains grown under similar conditions at 37°C (Fig. 1). Strain I failed to elaborate SEF17 fimbriae under any conditions tested, whereas strain 27655R, considered constitutive for SEF17 elaboration, did not elaborate SEF17 in broth at pH 4.65 but did so under all other conditions tested. Strain 27655S elaborated negligible SEF17, although detectable amounts were observed for surface agar growth near neutral pH only. Strains LA5, S1400 and E elaborated SEF17 in broth at pH 6.18 and above, but not at pH 5.09 or below, whereas surface-grown cultures elaborated SEF17 at these and the lower pH values tested. Elaboration of SEF21 and SEF14 fimbriae was negligible for all strains under all conditions at 20°C, although modest SEF21 elaboration by strains 27655R and S was noted (OD₄₅₀ 0.05–0.20).

Before recovery of bacteria for ELISA assays, pH measurements were made across the surface of inoculated and blank gradient plates to determine the effects of bacterial metabolism on the pH of the underlying medium. All strains tested raised the medium pH to 8.40 where confluent growth was visible (Table 2).

Colony morphology on CFA agar gradients

Allen-Vercoe et al. [43] noted that strains 27655R, LA5, E and S1400 generated a convoluted colonial morphology when grown at 20°C on unmodified CFA agar for extended periods (3–5 days) and similar morphological changes were observed here for these strains and for strain E (Fig. 2), but not strain I (data not shown). These experiments demonstrated for the first time that colonial morphology was dependent upon the initial pH of the agar surface growth medium with, for example, smooth colonies present at an initial pH 4.40 and convoluted at pH 5.80 (Fig. 2).

Discussion

Enteritidis strains E, I, LA5, S1400 and 27655S grown as planktonic cells in CFA broth at 37°C elaborated copious amounts of SEF14 fimbriae, elaboration of which decreased significantly at the lowest initial pH value (pH 4.04), observations broadly in line with those of McDermid et al. [49]. Although elaboration of SEF14 on the surface of agar was less than that for planktonic growth, increasing acidification of the underlying medium increased their elaboration. The differing patterns of expression between planktonic and surface agar growth indicated that surface contact was important in the regulation of SEF14 elaboration. Furthermore, and taking account of the obvious requirement for elevated temperature for SEF14 elaboration, it may be argued that at least three environmental signals, namely temperature, initial pH and surface contact, regulate SEF14 elaboration. How these observations relate to the nutritional regulation noted by Thorns et al. [37] is the subject of current genetic investigation [49]. The question arises as to whether contact with animate surfaces, such as gut epithelium, induces the same response by Enteritidis,
Table 1. Elaboration of flagella and fimbriae by six strains of *Salmonella Enteritidis* grown in planktonic or surface culture with defined conditions of pH and temperature

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>pH</th>
<th>Growth mode</th>
<th>LAS</th>
<th>S1400</th>
<th>E</th>
<th>I</th>
<th>27655R</th>
<th>27655S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>37</td>
<td>Broth</td>
<td>0.330 (0.036)</td>
<td>0.449 (0.038)</td>
<td>0.436 (0.028)</td>
<td>0.426 (0.027)</td>
<td>1.007 (0.017)</td>
<td>0.252 (0.006)</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>Surface</td>
<td>1.140 (0.070)</td>
<td>1.000 (0.051)</td>
<td>0.840 (0.034)</td>
<td>1.661 (0.057)</td>
<td>1.280 (0.056)</td>
<td>0.929 (0.028)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Broth</td>
<td>1.886 (0.199)</td>
<td>1.710 (0.084)</td>
<td>1.542 (0.163)</td>
<td>1.297 (0.172)</td>
<td>1.964 (0.046)</td>
<td>0.965 (0.067)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Surface</td>
<td>1.560 (0.120)</td>
<td>1.960 (0.149)</td>
<td>1.920 (0.177)</td>
<td>2.060 (0.433)</td>
<td>0.850 (0.033)</td>
<td>0.455 (0.011)</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>Broth</td>
<td>1.957 (0.084)</td>
<td>1.875 (0.098)</td>
<td>2.081 (0.047)</td>
<td>1.766 (0.057)</td>
<td>0.108 (0.006)</td>
<td>1.900 (0.040)</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>Surface</td>
<td>0.155 (0.013)</td>
<td>0.148 (0.008)</td>
<td>0.192 (0.008)</td>
<td>0.800 (0.181)</td>
<td>0.321 (0.061)</td>
<td>0.730 (0.021)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Broth</td>
<td>0.038 (0.009)</td>
<td>0.039 (0.007)</td>
<td>0.049 (0.010)</td>
<td>0.060 (0.010)</td>
<td>0.035 (0.012)</td>
<td>0.035 (0.012)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Surface</td>
<td>0.015 (0.012)</td>
<td>0.029 (0.012)</td>
<td>0.017 (0.015)</td>
<td>0.020 (0.002)</td>
<td>0.083 (0.018)</td>
<td>0.058 (0.008)</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>Broth</td>
<td>0.001 (0.002)</td>
<td>0.013 (0.023)</td>
<td>0.007 (0.008)</td>
<td>0.013 (0.005)</td>
<td>0.755 (0.052)</td>
<td>0.033 (0.005)</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>Surface</td>
<td>0.011 (0.013)</td>
<td>0.010 (0.004)</td>
<td>0.009 (0.005)</td>
<td>0.024 (0.011)</td>
<td>0.317 (0.022)</td>
<td>0.012 (0.007)</td>
</tr>
<tr>
<td></td>
<td>6.66</td>
<td>Broth</td>
<td>0.006 (0.001)</td>
<td>0.007 (0.002)</td>
<td>0.007 (0.012)</td>
<td>0.002 (0.003)</td>
<td>0.204 (0.017)</td>
<td>0.000 (0.000)</td>
</tr>
<tr>
<td></td>
<td>4.04</td>
<td>Broth</td>
<td>0.008 (0.002)</td>
<td>0.009 (0.010)</td>
<td>0.000 (0.000)</td>
<td>0.013 (0.004)</td>
<td>0.405 (0.008)</td>
<td>0.015 (0.009)</td>
</tr>
</tbody>
</table>

*Production of stated antigen by strain, OD_{A50} (SD)*
Table 1. Elaboration of flagella and fimbriae by six strains of *Salmonella* Enteritidis grown in planktonic or surface culture with defined conditions of pH and temperature

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>pH</th>
<th>Growth mode</th>
<th>LA5</th>
<th>S1400</th>
<th>E</th>
<th>I</th>
<th>27655R</th>
<th>27655S</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>6.57</td>
<td>Broth</td>
<td>0.310 (0.020)</td>
<td>0.271 (0.058)</td>
<td>0.382 (0.022)</td>
<td>0.014 (0.005)</td>
<td>0.605 (0.028)</td>
<td>0.017 (0.007)</td>
</tr>
<tr>
<td></td>
<td>6.18</td>
<td>Broth</td>
<td>0.388 (0.022)</td>
<td>0.365 (0.018)</td>
<td>0.169 (0.005)</td>
<td>0.008 (0.005)</td>
<td>0.670 (0.032)</td>
<td>0.019 (0.003)</td>
</tr>
<tr>
<td></td>
<td>5.09</td>
<td>Broth</td>
<td>0.016 (0.008)</td>
<td>0.001 (0.002)</td>
<td>0.017 (0.003)</td>
<td>0.008 (0.002)</td>
<td>0.723 (0.022)</td>
<td>0.022 (0.001)</td>
</tr>
<tr>
<td></td>
<td>4.65</td>
<td>Broth</td>
<td>0.017 (0.006)</td>
<td>0.005 (0.004)</td>
<td>0.017 (0.004)</td>
<td>0.005 (0.008)</td>
<td>0.575 (0.024)</td>
<td>0.013 (0.005)</td>
</tr>
<tr>
<td>20</td>
<td>6.57</td>
<td>Surface</td>
<td>0.199 (0.006)</td>
<td>0.286 (0.027)</td>
<td>0.287 (0.036)</td>
<td>0.006 (0.006)</td>
<td>0.379 (0.009)</td>
<td>0.216 (0.009)</td>
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<tr>
<td></td>
<td>6.18</td>
<td>Surface</td>
<td>0.220 (0.009)</td>
<td>0.345 (0.020)</td>
<td>0.201 (0.019)</td>
<td>0.004 (0.004)</td>
<td>0.441 (0.023)</td>
<td>0.095 (0.013)</td>
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<tr>
<td></td>
<td>5.09</td>
<td>Surface</td>
<td>0.317 (0.023)</td>
<td>0.268 (0.023)</td>
<td>0.133 (0.003)</td>
<td>0.006 (0.006)</td>
<td>0.488 (0.043)</td>
<td>0.060 (0.002)</td>
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<tr>
<td></td>
<td>4.65</td>
<td>Surface</td>
<td>0.168 (0.017)</td>
<td>0.104 (0.004)</td>
<td>0.091 (0.009)</td>
<td>0.000 (0.000)</td>
<td>0.020 (0.010)</td>
<td>0.066 (0.007)</td>
</tr>
</tbody>
</table>

**SEF21 fimbriae**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>pH</th>
<th>Growth mode</th>
<th>LA5</th>
<th>S1400</th>
<th>E</th>
<th>I</th>
<th>27655R</th>
<th>27655S</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>6.66</td>
<td>Broth</td>
<td>0.138 (0.014)</td>
<td>0.087 (0.010)</td>
<td>0.176 (0.061)</td>
<td>0.197 (0.018)</td>
<td>0.250 (0.038)</td>
<td>0.097 (0.027)</td>
</tr>
<tr>
<td></td>
<td>6.10</td>
<td>Broth</td>
<td>0.194 (0.035)</td>
<td>0.117 (0.015)</td>
<td>0.205 (0.001)</td>
<td>0.217 (0.026)</td>
<td>0.320 (0.048)</td>
<td>0.054 (0.017)</td>
</tr>
<tr>
<td></td>
<td>5.09</td>
<td>Broth</td>
<td>0.076 (0.008)</td>
<td>0.057 (0.003)</td>
<td>0.107 (0.010)</td>
<td>0.161 (0.011)</td>
<td>0.179 (0.015)</td>
<td>0.071 (0.008)</td>
</tr>
<tr>
<td>37</td>
<td>4.65</td>
<td>Broth</td>
<td>0.170 (0.005)</td>
<td>0.077 (0.004)</td>
<td>0.086 (0.007)</td>
<td>0.089 (0.005)</td>
<td>0.118 (0.016)</td>
<td>0.064 (0.009)</td>
</tr>
</tbody>
</table>

*Production of stated surface antigen was quantified by ELISA (see Materials and methods). Results are recorded as OD$_{450}$ (SD) from three different tests.*
Fig. 1. Transmission electron micrograph of negatively stained *Salmonella Enteritidis* strain 276553 grown confluently on CFA agar at an initial pH of 6.57 at 20°C for 24 h. Bar = 3 μm.

Table 2. pH measurement across the surface of inoculated and unoinoculated plates

<table>
<thead>
<tr>
<th>Distance across gradient plate (mm)</th>
<th>Initial pH across gradient plates</th>
<th>pH* across blank plate</th>
<th>pH* across inoculated plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>7.20</td>
<td>7.14</td>
<td>8.56</td>
</tr>
<tr>
<td>30</td>
<td>6.87</td>
<td>6.84</td>
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<tr>
<td>50</td>
<td>5.24</td>
<td>5.05</td>
<td>8.42</td>
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<tr>
<td>70</td>
<td>4.41</td>
<td>4.24</td>
<td>8.21</td>
</tr>
<tr>
<td>90</td>
<td>3.92</td>
<td>3.79</td>
<td>4.29</td>
</tr>
<tr>
<td>110</td>
<td>3.71</td>
<td>3.58</td>
<td>3.68</td>
</tr>
</tbody>
</table>

*After incubation at 37°C for 24 h. Note: visible growth boundary was 80 mm across plate towards lowest pH values (see also Fig. 2).

but evidence has not yet been obtained for a role for SEF14 in adherence and invasion in HEp-2 cell culture [38] and chick gut explant [Allen-Vercoe and Woodward, unpublished observations], although a role in transovarian transmission has been suggested [50].

SEF17 fimbriae were elaborated at 20°C but not at 37°C in both planktonic and surface culture, findings in broad agreement with those of Dibb-Fuller *et al.* [42]. Notable exceptions were strain I, an *rpoS* mutant, which failed to express the antigen under any circumstances, and strain 27655R which was considered to be constitutive for SEF17 [26]. However, SEF17 was elaborated on surface culture at lower initial pH values than in planktonic culture and it may be argued that surface contact is one environmental signal that contributes to the regulation of this fimbrial antigen. That SEF17 fimbriae are elaborated at low pH at ambient temperatures and are highly hydrophobic might lend support to the suggestion that this may be a protective response against environmental extremes [26]. Strain 27655R did not elaborate SEF17 in planktonic growth at 20°C at pH 4.65, indicating that a constitutive mutation was not the basis for the aberrant expression. The mutation seemed to have a pleiotropic effect upon the expression of other surface antigens, that was ameliorated partially in strain 27655S, presumably by a second compensatory mutation. This is the subject of further genetic analysis.

When test bacteria were diluted and plated on acid gradient plates to obtain single colonies, different colonial morphologies were observed at distinct initial pH values. Whilst SEF17 expression is essential for convoluted colonies [43], the many morphologies identified were probably too diverse to be related to the presence or absence of SEF17 alone, and is probably associated with production of an extracellular matrix (ECM) which has been observed surrounding bacteria in convoluted colonies [51–53]. Habituation at an initial pH induced responses which generated a specific colony morphology that persisted as the pH changed. Whether up-regulation of SEF17 and ECM is
ELABORATION OF FIMBRIAE AND FLAGELLA BY *S. ENTERITIDIS*

![Diagram showing pH changes across a gradient plate](image)

**Fig. 2.** Morphological variation shown by *Salmonella* Enteritidis strain E grown as single colonies on neutral-pH 4 gradient plates at 20°C for 5 days. Bar = 5 mm.

Elaboration of SEF21 fimbriae by Enteritidis was poor under all the conditions tested in these experiments, with the notable exception of strain 27655R, whilst previous studies [25] demonstrated that optimal expression was shown with planktonic growth in heart infusion broth at 37°C for 48–72 h. The role of surface contact in their elaboration is unclear, but there is evidence of SEF21 expression *in vivo* in contact with gut epithelial cells [55].

Hyper-flagellation has been noted for serotypes Typhimurium [56], Enteritidis [43] and Pullorum, which is traditionally regarded as non-motile [57], with the abundance of flagella dependent upon agar concentration, temperature and carbohydrate availability. The experimental data from the present study lend weight to this body of information, although the mode of growth and initial pH were of marginal influence. For Typhimurium, flagella contribute to invasion and intracellular survival [18, 58, 59] in murine and chick models and it may be assumed that similar roles are performed by the flagella of Enteritidis. Whether variability in abundance of flagella influences pathogenesis, and what role environmental signals play in the biogenesis of flagella, requires further study. We argue that the surface architecture of Enteritidis was influenced by an agar surface on which to grow, a
suggestion supported by Harshney and Matsuyma [56] for Typhimurium. There are similarities between hyper-flagellation and elaboration of SEF17 and ECM by Salmonella strains and the swarming of Proteus mirabilis [60] which may impact upon pathogenesis, particularly routes of infection.

We thank Stefan Farelley for assistance with electron microscopy. We acknowledge the very helpful discussions with Professor Tom Humphrey, Emma Allen-Vercoe and others in the BA3 team; and funding from the Ministry of Agriculture Fisheries and Food.

References


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