ROLE OF DNA AND BACTERIOPHAGE IN *CAMPYLOBACTER* AUTO-AGGLUTINATION

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SUMMARY. Auto-agglutinated and non-agglutinated cells of *Campylobacter jejuni* and *C. coli* were examined by transmission electronmicroscopy in phosphotungstate negative stain. Agglutination was induced by three factors (1) extracellular DNA, (2) an aggregated protein, probably a bacteriophage precursor, and (3) free phage-tail sheaths. Auto-agglutinated cells were often “leaky,” with a mantle of adhering DNA. About 80% of the auto-agglutinated cells could be resuspended after treatment with DNAase. Flagella were loosely embedded in protein aggregates, especially in phage-infected cultures. They were clumped in a side-by-side arrangement by free phage-tail sheaths. These findings suggest that auto-agglutination could be minimised in suspensions of organisms intended for use in agglutination tests by harvesting early logarithmic-phase cells containing no more than a low phage population. The most common *C. jejuni* phage had a contractile tail, a head diameter of 60–70 nm, and an overall length of 180–210 nm. A phage isolated from *C. jejuni* strain 1590 was morphologically identical with *C. coli* phage.

INTRODUCTION

Auto-agglutination interferes with the serotyping of some *Campylobacter jejuni* isolates in simple tube tests (Butzler, 1978). Its cause has not been studied, but various practical means have been used to circumvent the problem, e.g., the use of (1) “stable” comma-shaped cells obtained by differential centrifugation (Lussier, 1962), (2) autoclaved cells (Berg, Jutila and Firehammer, 1971), (3) alternative tests such as line-rocket immunoelectrophoresis (R-LIE) or co-agglutination with antibody-coated reagent staphylococci (COA; Kosunen, Danielsson and Kjellander, 1980), and (4) cells preincubated with DNAase (Lior *et al.*, 1982). Our objective was to determine the cause of auto-agglutination by examining whole cells by transmission electronmicroscopy. Because our preliminary observations implicated a bacteriophage component or precursor (Ritchie, Bryner and Foley, 1982), we compared the morphology of representative phages from *C. jejuni* and *C. coli*.

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MATERIALS AND METHODS

Bacterial strains. The nomenclature used is that recommended by Skerman, McGowan and Sneath (1980). *C. jejuni* strains 657, 917 and 958 were isolated from aborted ovine fetuses, strains 1398, 1425 and 1460 from cases of human enteritis, and strains 1590 and 1591 from rabbit faeces. *C. coli* strains 425, 1380 and 1491 were isolated from a porcine kidney, an aborted porcine fetus, and porcine faeces, respectively. Cells were grown at 37 or 43°C on Mueller Hinton Agar (MH; Difco Laboratories, Detroit, MI) in O₂ 5%, CO₂ 10% and N₂ 85% for 18 h or more (Bryner, Ritchie and Foley, 1982). When needed, larger cell masses were grown in shaker cultures of Albimi Brucella Broth (Difco Laboratories) as previously described (Bryner, Frank and O'Berry, 1962). Inocula for shaker cultures were from MH plates.

Electronmicroscopy and specimen treatment. For negative staining in neutralised phosphotungstate (PTA), the method of Brenner and Horne (1959) as modified by Ritchie and Fernelius (1969) was used. To minimise the physical disturbance of any structure that might be responsible for adherence of the bacteria to each other, each sample was applied to a carbon-collodion grid with a 5-mm platinum loop instead of a nebuliser. For this manipulation, the grid was placed on an absorbent paper towel and the charged loop was applied in a single motion so that the material spread evenly and absorption was uniform from the grid edge. Direct immuno-electronmicroscopy (DIEM) was done by incubating suspensions of bacteria and their phages with excess antiserum overnight at 4°C, centrifuging at 7000 *g* for 30 min, resuspending the pellets in distilled water, and staining negatively with PTA. Enzymic digestion of shaker cultures was done with DNAase (ICN Nutritional Biochemicals, Cleveland, OH) 10–20 µg/ml at 37°C for 1–4 h.

RESULTS

Early logarithmic-phase, non-agglutinating cultures contained turgid cells with rugose surfaces (fig. 1). Ultrastructurally, both *C. jejuni* and *C. coli* resembled *C. fetus* (Werner, Levy and Spurlock, 1961; Ritchie, Keeler and Bryner, 1966) and recently examined isolates of *C. jejuni* from man (Pead, 1979). No surface appendages other than flagella were present, nor were the flagella sheathed.

When auto-agglutinated cells, usually from 48-h cultures, were examined, > 40% were seen to be either "leaky" or disrupted. Leakiness was indicated by a flaccid appearance and some internal staining by the PTA. Most leaky cells had a closely

FIG. 1.—*Campylobacter jejuni* strain 657. Typical non-agglutinating logarithmic-phase cells with rugose surface and non-sheathed flagella; no other surface appendages are present. Electronmicrograph (EM).

adhering mantle of an amorphous, streaming material (fig. 2). This could be removed by treatment with DNAase (fig. 3). DNAase did not, however, completely reverse auto-agglutination: *c.* 20% of the agglutinated cells of strain 958 could not be permanently resuspended after DNAase digestion. Ruptured cells often released masses of an aggregated protein that were generally associated with agglutinated flagella or flagellate cells (figs. 4 and 5). This substance was plentiful only in phage-bearing cultures. When present, free phage-tail sheaths (usually "triggered") were almost always found adhering to and parallel with a flagellum (fig. 6), thus inducing neighbouring flagella to aggregate in a linear arrangement (fig. 7).

Because of these unexpected findings, we compared the features of representative *C. jejuni* and *C. coli* phages. The most common *C. jejuni* phage had a contractile tail and was usually found unattached to cells or wall fragments (fig. 8*a*). Its dimensions were: head diameter, 60–70 nm; untriggered tail, 120–140 nm $\times$ 16–22 nm; triggered tail sheath, 50–60 nm $\times$ 25–36 nm; and needle, 120–140 nm $\times$ 9–10 nm. Measurement of the tail-sheath width was difficult because of an indistinct outer component (fig. 8*b*).

FIG. 2.—*C. jejuni* strain 1398. Auto-agglutinating cells with mantle of streaming amorphous DNA (arrowed); leakiness is indicated by intracellular accumulation of phosphotungstate. EM.

FIG. 3.—*C. jejuni* strain 917. Auto-agglutinating cells after DNAase treatment; note absence of the DNA mantle. EM.

FIG. 4.—*C. jejuni* strain 958. Entangled agglutinated flagella commonly seen in phage-infected auto-agglutinating cultures. EM.

FIG. 5.—*C. jejuni* strain 958. Portion of a flagellar clump showing the aggregated protein (arrowed) presumed to induce agglutination. EM.

FIG. 6.—*C. jejuni* strain 958. Portion of a single flagellum (F) with parallel, adhering phage-tail sheaths; note the fibrous, indistinct bridging between the tail's outer layer and the flagellar surface. EM.

FIG. 7.—*C. jejuni* strain 958. Typical flagellar agglutination with numerous phage-tail sheaths attached to the flagellum. EM.

FIG. 8a and 8b.—*C. jejuni* strain 958. (a) Most common phage type with a contractile tail and isometric head; (b) triggered phage with empty head and indistinct outer-sheath layer. EM.

FIG. 9a and 9b.—*C. coli* strain 1491. (a) Typical intact phage with contractile tail; note knobby outer components at the tail's distal end. A similar phage was isolated from *C. jejuni* strain 1590. (b) Phage lacking a tail sheath but showing a characteristic distal collet (arrowed). EM.

Baseplate and tail-fibre assemblies were either absent or lacked sufficient structural integrity to be detected.

The *C. coli* phage examined in our study was from strains 1380 and 1491; it was similar to the contractile-tail type described by Fletcher (1965) but was larger and displayed a more angular head profile (fig. 9a) than the commonest *C. jejuni* phage. Its dimensions were: head diameter, 100–120 nm; untriggered tail, 140–160 nm $\times$ 18–21 nm; triggered tail sheath, 60–70 nm $\times$ 28–33 nm; and needle, 130–150 nm $\times$ 9–11 nm. Its baseplate and tail fibre assembly often had a large collet (fig. 9b). A phage isolated from *C. jejuni* strain 1590 was indistinguishable from the *C. coli* phage. By DIEM, none of its structural components appeared to share any antigens with the *C. jejuni* phage.

DISCUSSION

Auto-agglutination appeared to be facilitated by three factors released from "leaky" or disrupted cells: (1) nascent DNA, (2) an adventitious aggregated protein, and (3) free phage-tail sheaths, when present. Extruded DNA accounted for most of the auto-agglutination in cultures in the stationary phase of growth because c. 80% of the agglutinated cells could be resuspended after DNAase treatment. The percentage of cells capable of being resuspended will probably vary from one auto-agglutinating culture to another, but our results support the rationale put forward by Lior *et al.* (1982) for their use of DNAase treatment, when necessary, in the slide agglutination test. The amount of extracellular DNA, and hence the extent of auto-agglutination, is probably controlled by the average cell age and by the activity of phages when present. The loop sampling method facilitated the detection of adhering DNA and the effects of digestion by DNAase. The adventitious aggregated protein associated with flagella probably represented a secondary auto-agglutinating factor. It may have accounted for the incomplete reversal of agglutination by DNAase. Because this protein was plentiful only in phage-infected cultures, we believe that it was a phage precursor. Free phage-tail sheaths, although uncommon in most cultures, had a striking affinity for the flagella. The parallel arrangement of the two structures was probably mediated by the sheaths' outer protein. The latter may have had as its unassembled precursor the adventitious aggregated protein. Fletcher (1965) observed an association of *C. coli* phage with flagella but the phage-tail sheath did not appear to be directly involved. Recognition of the three auto-agglutinating factors suggests that stable campylobacter suspensions for use in agglutination tests could be prepared from logarithmic-phase phage-free cultures.

Although *C. fetus* cultures do not auto-agglutinate, it is appropriate to summarise our observations on this species. None of the precipitating factors described here have been observed in *C. fetus* cultures, whether phage-infected or not. Phages of *C. fetus* lack a tail sheath (Bryner *et al.*, 1973). This probably accounts for our inability to detect a protein with comparable flagellar affinity. The absence of adhering extracellular DNA is unexplained. However, we found that phage infection usually led to rapid and extensive lysis.

The taxonomic significance of our observation of a *C. coli*-type phage in *C. jejuni* strain 1590 has not been assessed. Restriction endonuclease analyses of the cellular and phage DNA might provide an insight into this interesting anomaly.

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