

RESEARCH NOTE

Toxigenic and Invasive Capacities: Possible Pathogenic Mechanisms in *Arcobacter cryaerophilus*

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Arcobacter cryaerophilus, formerly known as aerotolerant *Campylobacter*-like microorganisms and *Campylobacter cryaerophila*, is a Gram negative curved or S shaped rod, able to grow in normal O₂ tension and optimal temperature of 30°C (P Vandamme, H Goosens 1992 *Zbl Bakt* 276: 447-452). It has been isolated from aborted animal fetuses, from tissues and feces from cattle, swine and poultry and also from human stool, abdominal content and blood as well as from surface water and sewage (S Neill et al. 1985 *Int J Syst Bacteriol* 35: 342-356, M Lambert et al. 1987 *J Clin Microbiol* 25: 706-713, W Tee et al. 1988 *J Clin Microbiol* 26: 2469-2473, S Stampi et al. 1993 *Epidemiol Infect* 110: 633-639). This isolation spectrum suggests a wide natural distribution and reflects the zoonotic character of *A. cryaerophilus*. However, the factors of pathogenicity and the clinical significance of this bacteria remains unknown.

The aim of this study was to determine the existence of toxigenic and invasive capacities in two strains of *A. cryaerophilus* isolated from an aborted bovine fetus (strain 7625) and from swine feces (strain 62C).

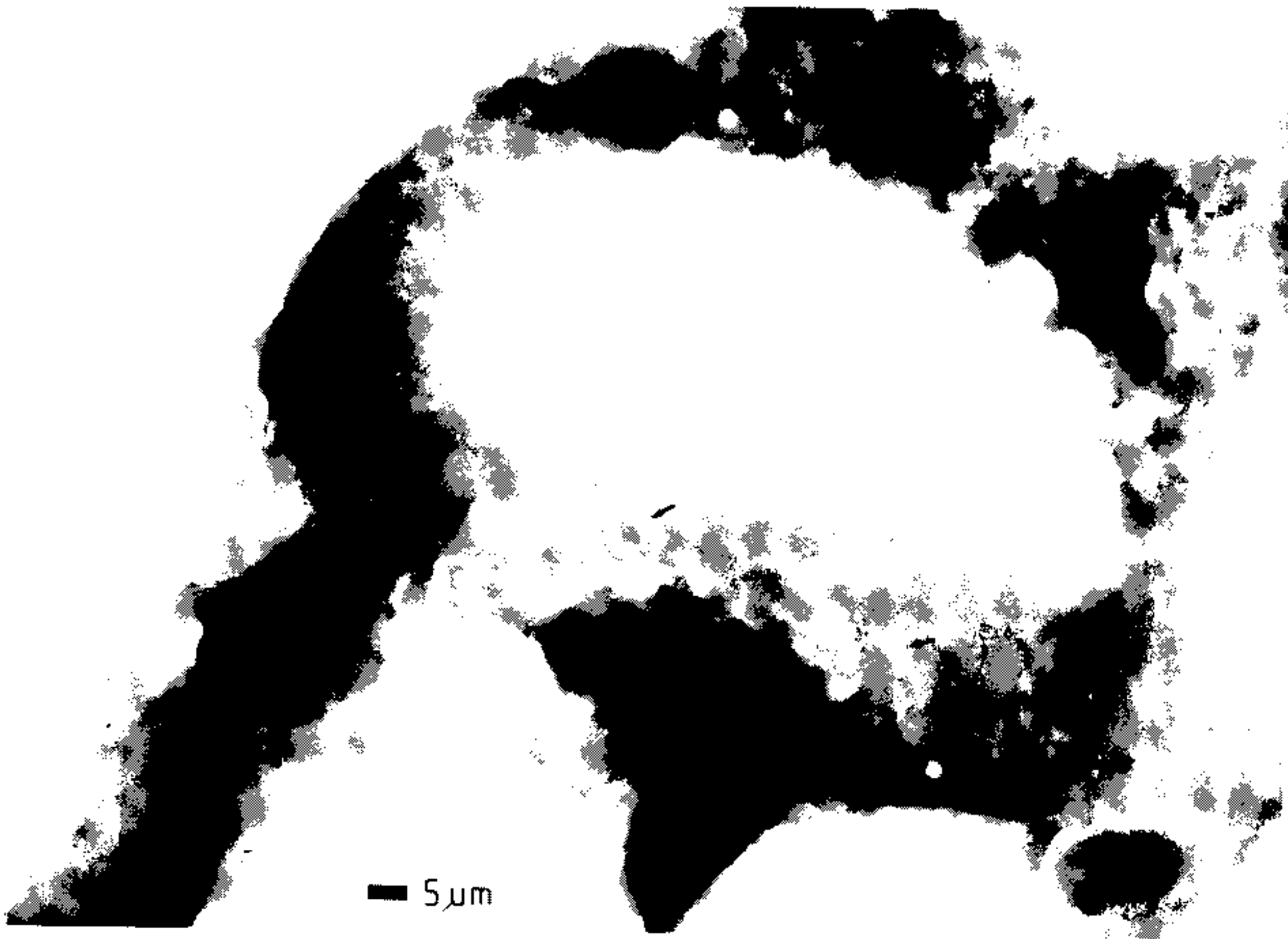
Both strains were isolated on blood agar using a filtration method (T Steele, S McDermontt 1984 *Pathology* 16: 263-265), phenotypically identified (Vandamme, Goosens *loc. cit.*) and maintained under freezing temperature (-35°C) until use.

The toxigenic capacity was determined by the rat ileal loop test (S Saha et al. 1988 *J Med Microbiol* 26: 87-91). A cell-free supernatant of each strain was prepared from a 72 hr culture in Brucella broth and 400 µl were inoculated individually into two ileal loops of adult Wistar rats. Sterile Brucella broth and the cell-free supernatant of a toxigenic *C. jejuni* strain were used as negative and positive control respectively. After 18 hr, the rats were killed by ether overdose and the ileum examined. Distention of the loops with fluid accumulation was considered as a positive result and electrolyte concentrations were measured in the intestinal fluids by standard flame spectrophotometry. The tests were done in two animals simultaneously and repeated three times.

Invasion assays were done in 18 hr old HEp-2 cells cultures (120,000 cells/ml). Four Leighton tubes containing a coverslip with the cell cultures were used for each strain. They were inoculated with 1 ml of the bacterial suspensions (10⁶ c.f.u) made in the same medium used for growing HEp-2 cells (RPMI medium with 10% fetal calf serum) and incubated during 3 hr (infection period), at 37°C in a CO₂ incubator. Following the washing (10X) of the tubes, 1 ml of the cell growing medium was added and the cell monolayers were reincubated for further 4 hr (multiplication period) under the same conditions mentioned above. Then, the tubes were washed three times and the coverslips with the cell monolayers were stained with 0.01% acridine orange in Gey's solution for 45 sec, rinsed with Hanks balanced salts solution, counterstained for 45 sec with 0.05% crystal violet in 0.15 N NaCl, rinsed again, mounted on slides and examined under epifluorescence microscopy. Internalized bacteria were seen as green-fluorescing curved rods (Fig.). Both strains produced distention of the ileal loops with fluid accumulation and enhanced electrolytes concentrations. The concentrations of Na⁺ and Cl⁻ (mEq/l) in the intestinal fluids produced by strains 7625 and 62C were 135 and 114, and 134 and 117 respectively whereas, in the negative control loop, they were 26 and 40. Toxin production in *C. jejuni* was previously demonstrated using this biological model (Saha et al. *loc. cit.*). Fluids and electrolyte excretion to the intestinal lumen of the rat was also demonstrated in *C. jejuni* (H Fernández et al. 1983 *Infect Immun* 40: 429-431), suggesting the presence of a toxigenic substance as described for other toxigenic bacteria (R Sullivan, T Asano 1971 *Am J Physiol* 220: 1793-1797, F Klipstein et al. 1976 *Infect Immun* 14: 1004-1010).

Invasion to HEp-2 cells was observed with both strains. In this study we used the same method described for *C. jejuni* (H Fernández 1983 Doc-

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HEp-2 cells showing (arrows) internalized *Arcobacter cryaerophilus*. Bar = 5 μm.

toral Thesis, Escola Paulista de Medicina) but using the acridine orange stain technique proposed by M Miliotis (1991 *J Clin Microbiol* 29: 830-831). This method showed to be useful to study invasion in *A. cryaerophila* as well as in *C. jejuni* (unpublished data).

This results suggest that toxigenicity and invasion could be pathogenic mechanisms by which *A. cryaerophilus* may produce disease. However, further studies are necessary to elucidate the pathobiology of this bacteria as well as their clinical and epidemiological significance. Moreover, specific

isolation media need to be developed, since most strains are sensitive to some of the antibiotics used in *Campylobacter* selective media (J Kiehlbauch et al. 1992 *Antimicrob Agents Chemother* 36: 717-722) and are unable to grow at 42°C. These facts delay the isolation of *A. cryaerophilus*, that may pass undetected (J Penner 1988 *Clin Microbiol Rev* 3: 157-172) in man and animals biological products in which more attention is given to other gram negative curved rods such as the well known *Campylobacter* species. Currently, studies focusing these aspects are in course in our laboratory.