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NOTES

Application of the Fortner Principle to Isolation of *Campylobacter* from Stools

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A simple biological technique for reducing oxygen tension which does not necessitate the use of conventional anaerobic equipment is described. We successfully applied this method to the isolation of campylobacters from stools.

Campylobacter jejuni (3, 7), corresponding to the *Campylobacter jejuni-Campylobacter coli* group of Véron and Chatelain (7) and to the *Campylobacter fetus* subsp. *jejuni* of Smibert (6), has recently become recognized as a common cause of diarrhea (1, 4, 5). It is a strict microaerophile which grows best at 42°C (6). Primary isolation of this organism from stools may be made on a selective medium containing antibiotics, as described by Skirrow (5), which is incubated at 42°C under reduced oxygen tension. An increased carbon dioxide tension is also considered to be required for growth (6). Smibert (6) recommends an atmosphere of about 6% oxygen, 10% carbon dioxide, and 85% nitrogen for the optimal growth of these organisms. The reduced oxygen tension may be achieved by evacuating two-thirds of the air from an anaerobic jar (without a catalyst). The final optimal gaseous environment is made up by replacing the evacuated air with carbon dioxide (to a final concentration of 10%) and nitrogen (to avoid negative pressure in the jar).

This report describes an alternate method for achieving a reduced oxygen tension, based on the Fortner principle (2), which utilizes the ability of a rapidly growing facultative anaerobe to reduce the oxygen tension in a closed system, thus making possible the growth of oxygen-sensitive organisms such as campylobacters.

In our initial experiment, a strain of *C. jejuni* was streaked onto one half of a blood agar plate, and a strain of *Proteus rettgeri* was streaked onto the other half. The plate was then hermetically sealed with autoclave tape and incubated at 42°C. After 48 h of incubation, it was found that *C. jejuni* readily grew in the presence of *P. rettgeri* (Fig. 1). Growth of *C. jejuni* occurred in a similar fashion when the *P. rettgeri* was replaced by a strain of either *Staphylococcus aureus, Escherichia coli, Klebsiella aerogenes*, or *Pseudomonas aeruginosa*, respectively. However, growth of *C. jejuni* did not occur if another organism was not concomitantly cultured onto the same blood agar plate.

In the second experiment, we applied the same technique to the isolation of *C. jejuni* from stools. The medium used was a Skirrow-type selective medium (4) containing 5.0 μg of polymyxin B sulfate per ml, 10.0 μg of vancomycin per ml, and 5.0 μg of trimethoprim lactate per ml. One half of the plate was streaked with a stool specimen known to be culture positive for *C. jejuni*. The opposite half was streaked with a strain of *P. rettgeri* known to be resistant to the concentrations of the three antibiotics used in the selective medium. After being sealed with autoclave tape, the plate was incubated for 48 h at 42°C. *C. jejuni* was isolated from the stool by this method (Fig. 2).

In the third experiment, the technique was modified so that the stool was cultured onto a whole plate of selective medium, and the *P. rettgeri* was cultured onto a separate whole blood agar plate. Both plates were placed in a polythene bag (18 by 20 cm) equipped with a reversible airtight seal—"baggy" (available commercially as Ziploc storage bags, Dow Chemical of Canada Ltd.). After manually expelling as much air as possible from the baggy, it was carefully sealed (Fig. 3) and incubated at 42°C for 48 h. *C. jejuni* was again recovered from the stool sample (Fig. 4).

Finally, the baggy method was evaluated for its applicability to the routine isolation of *C. jejuni* from stools. Thirty stool samples known to be positive for *C. jejuni* were cultured in parallel by both the baggy method and our conventional method for achieving reduced oxygen tension. Our conventional method consists of evacuating two-thirds of the air from an anaerobic jar (without catalyst) and replacing the

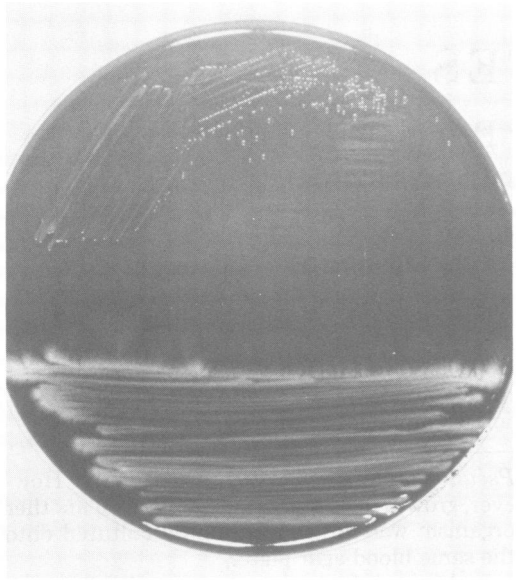

FIG. 1. *Blood agar plate with a strain of P. rettgeri streaked onto one half and a strain of C. jejuni streaked onto the opposite (top) half of the plate. The plate was sealed with autoclave tape and incubated at 42°C. Growth of both organisms was noted after 48 h of incubation, as shown.*

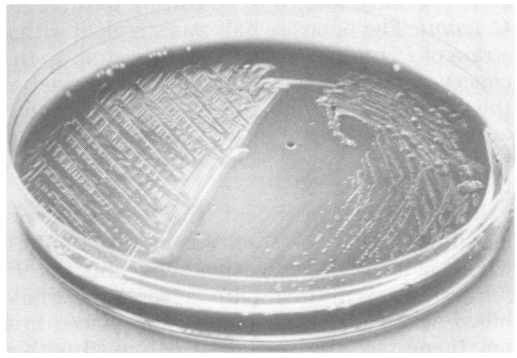

FIG. 2. *Skirrow-type selective medium with P. rettgeri streaked onto one half and a stool specimen streaked onto the opposite half of the plate. The plate was sealed with autoclave tape and incubated at 42°C. After 48 h of incubation, a heavy growth of C. jejuni resulted, as shown (right half of plate).*

evacuated air with carbon dioxide (nitrogen is not routinely used in our laboratory). After 48 h of incubation at 42°C, it was found that *C. jejuni* was isolated from all 30 stool samples by both methods. We found it helpful to add a piece of moist cotton wool to the baggy before incubation. The increased moisture resulted in the swarming type of campylobacter colony that was easier to recognize. It should be noted that all stool isolates were gram-negative curved or spiral organisms which showed a characteristic corkscrew-like darting motility when seen under a phase-contrast microscope. All isolates were strictly microaerophilic, oxidase, catalase, and nitrate positive, and inert to carbohydrates. They grew at 42°C but not at 25°C and were sensitive to 30 μg of nalidixic acid per ml.

We conclude that the atmosphere generated by the use of the Fortner principle is suitable for the growth of *C. jejuni*. Such an atmosphere is likely to have a reduced oxygen tension and an

FIG. 3. *Skirrow-type selective medium streaked with a stool specimen and a blood agar plate streaked with P. rettgeri in an airtight polythene bag with a reversible seal. Note that as much air as possible is expelled from the bag before careful sealing.*

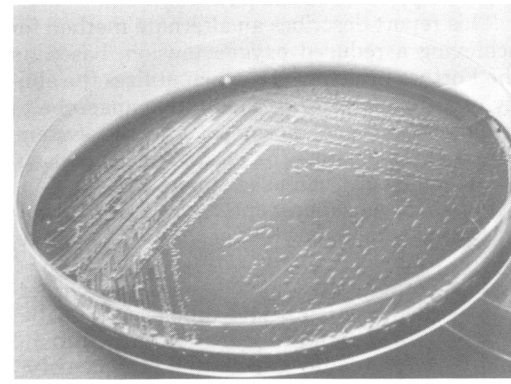

FIG. 4. *Heavy growth of C. jejuni from a stool sample cultured onto a Skirrow-type selective medium which was incubated by the baggy method for 48 h at 42°C.*

increased carbon dioxide tension resulting from the metabolism of the facultative anaerobic organism.

The baggy method may be successfully applied to the isolation of *C. jejuni* from stools. This simple and inexpensive method eliminates the use of anaerobic jars, gas cylinders, and vacuum pumps and is readily applicable to routine laboratories which lack such equipment.

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