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Effect of Moisture Content of the Medium on Colony Morphology of *Campylobacter fetus* subsp. *jejuni*

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Reduction in the moisture content of the medium produced a profound effect on the colony morphology of *Campylobacter fetus* subsp. *jejuni*. Fresh medium produced flat, grayish, spreading colonies with an irregular shape and watery appearance. Plates that were incubated at 30°C for 48 h produced round, convex, butyrous colonies with an entire edge. Plates incubated at 30°C for 24 h before inoculation produced colonies of an intermediate nature; they were round and raised, but not convex, and slightly watery or mucoid in nature. This marked effect produced by moisture content of the medium was reproducible and may account for the variation in colonies observed by other investigators.

Many laboratories have begun culturing for *Campylobacter fetus* subsp. *jejuni*. Several different media have been described for culturing this organism (1, 3, 7), and several methods have been proposed for obtaining the appropriate atmospheric conditions (1, 4, 6, 10), but the optimum growth conditions have not yet been entirely defined.

Two distinct colony types have been described. One is round, convex, and smooth with an entire edge; the other is flat, grayish, and watery, with a tendency to spread (8). During the time we have been culturing for this organism in our laboratory, we have noticed that the type of colony obtained seems to be related to the freshness of the medium. Colonies grown on fresh plates generally exhibit more spreading than colonies grown on older medium. It seemed that this was related to the moisture content of the medium; therefore, we conducted an experiment to test this hypothesis.

Fresh Columbia blood agar plates with 5% sheep blood were prepared. One-third of the plates were incubated at 30°C for 48 h; one-third were incubated at 30°C for 24 h; and the remaining third were refrigerated at 4°C and then dried briefly at 30°C to remove excess surface moisture before inoculation. All plates were held in the dark to avoid any effect of peroxides that might be produced by exposure to light (9).

Ten different isolates of *C. fetus* subsp. *jejuni* were then inoculated onto the plates and incubated in a Torbal jar at 42°C for 48 h. The necessary microaerophilic atmosphere was supplied by evacuating air from the jar and replacing it with a mixture of 5% oxygen, 10% carbon dioxide, and 85% nitrogen. The strains were previous clinical isolates which had been stored at −70°C in skim milk.

The results are illustrated in Fig. 1. Colonies grown on plates dried for 48 h were circular and convex with an entire edge (Fig. 1A); colonies on the fresh plates were spreading and flat (Fig. 1C). Plates dried for only 24 h produced colonies of an intermediate nature (Fig. 1B). When the circular, convex colonies were transferred to new, fresh plates, they produced the spreading type of morphology, indicating that this effect was reversible. These experiments were repeated several times with similar results.

The possibility that moisture added to the jar could reverse this effect was tested by incubating dried plates in a jar with a wet paper towel in the bottom. The colonies did not spread, however. Thus, increased moisture in the atmosphere cannot reverse the effect of low moisture in the medium.

These results show that the moisture content of the medium has a profound effect on the colony morphology of *C. fetus* subsp. *jejuni*. This may well account for the colony variation which has been observed by others (5, 8). Small variations in the moisture content could affect the type of colonies encountered in different laboratories, or those observed from day to day within the same laboratory. Of course, it is also possible that other factors, such as concentration of agar, the state of the nutrients, or buildup of toxic products (9), could have a similar effect. These possibilities await further investigation.

The biochemical or ultrastructural basis for this phenomenon is not readily apparent. Smi-

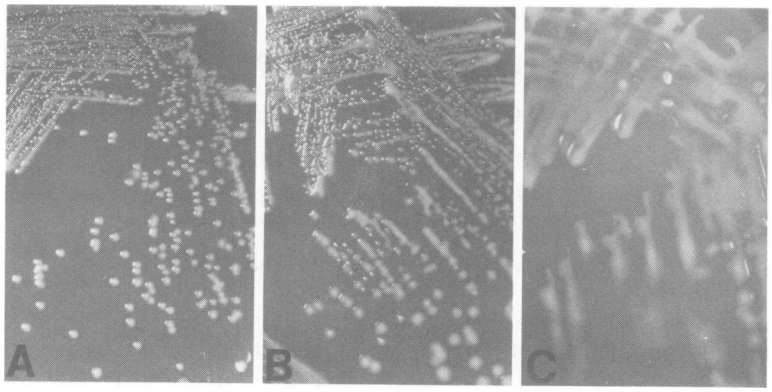

FIG. 1. *Colonies of C. fetus subsp. jejuni grown on media of different moisture contents. (A) Plates dried at 30°C for 48 h produced colonies that were circular and convex, with an entire edge. (B) Plates dried at 30°C for 24 h produced colonies that were slightly larger and more moist. (C) Fresh plates, dried briefly to remove surface moisture, produced flat, watery colonies with an irregular shape and a tendency to spread.*

bert (8) examined the ultrastructure of organisms from different types of colonies, but could detect no difference. Although Bryner et al. (2) concluded that dissociation from smooth to rough forms accounted for the different types of colonies they observed, this appears to be a different type of phenomenon since the variation we have observed seems to be totally controlled by the treatment of the medium.

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