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Utilization of Exogenous Siderophores by *Campylobacter* Species

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The availability of iron to potentially pathogenic bacterial strains is restricted by the iron-binding proteins of the host. In this study, we examined 40 strains of *Campylobacter* species grown under iron-limiting conditions. While the strains produced no detectable siderophores, all the isolates freely utilized exogenous siderophores produced by other organisms as iron carriers. These data suggest that the use of an exogenous siderophore (either purified or present in a coinfecting microorganism) may be important in developing a suitable laboratory model for campylobacteriosis.

Iron is essential for the growth of nearly all bacteria (13, 24), but its availability is complicated by its extreme insolubility at neutral and alkaline pHs (6). To acquire the necessary iron, aerobic microorganisms produce high-affinity compounds which bind and solubilize iron and transport it across the cell membrane. The iron-binding compounds are collectively termed siderophores (6, 7). Microbial siderophores can be divided into two chemical types: phenolates and hydroxymates (11, 12).

Enterobactin, a phenolate-type siderophore, is synthesized by *Salmonella typhimurium* (19, 25), *Escherichia coli* (14, 15), *Enterobacter aerogenes* (14, 15), and *Shigella sonnei* (18). Related compounds are also produced by other enteric bacteria (19). Some examples of hydroxymate siderophores include rhodotorulic acid produced by *Rhodotorula pilimanae* (1), ferrichrome made by *Ustilago sphaerogena* (10). and schizokinen produced by *Bacillus megaterium* (3).

Siderophores are important to understand because the growth of pathogenic bacteria in vivo depends upon their capacity to obtain the iron firmly held by substances such as transferrin and lactoferrin (2, 4, 9, 23). These abilities to produce siderophores and acquire iron permit bacterial multiplication in animals and may thus be regarded as a virulence factor (4, 13, 17, 21).

Transport of the iron-siderophore complex into the cell is possible because of specific membrane receptors. Some bacteria such as *S. typhimurium* possess receptors for siderophores produced by other organisms and have been shown to utilize schizokinen, ferrichrome, or other hydroxymates for the uptake of iron (7). *Shigella flexneri* also uses siderophores produced by other bacteria (16).

Campylobacter species do not seem to produce any siderophore which can use iron, normally complexed with transferrin in the host. The purpose of this study was to investigate the ability of *Campylobacter jejuni, C. coli,* and *C. laridis* to utilize exogenously supplied siderophores for growth on iron-deficient media with methods described elsewhere (9).

MATERIALS AND METHODS

Bacterial strains. A total of 20 *C. jejuni* strains and 10 strains each of *C. coli* and *C. laridis* isolated from cases of sporadic diarrhea were used in this study. Bacterial suspensions were adjusted to turbidity with a no. 2 McFarland standard and diluted 1:20. Saturated swabs were used to inoculate test media.

Iron-deficient medium. Medium A was prepared by adding 1 part of presterilized bovine serum (GIBCO Diagnostics, Madison, Wis.) with 3 parts of previously autoclaved GC medium base (Difco Laboratories, Detroit, Mich.). The serum was mixed thoroughly in the medium and incubated for 1 h at 56°C to inactivate the complement and allow time for the binding of all free iron to transferrin (5).

Medium B was prepared by adding Desferal mesylate (CIBA-GEIGY Corp., Summit, N.J.) to previously autoclaved GC medium base in the final concentration of 25 μM (8).

Siderophores. Enterobactin, ferrichrome (iron-free), and rhodotorulic acid were prepared as 0.001% solutions in sterile, glass-distilled water. Paper disks (6 mm) were impregnated with 20 μl of each solution immediately before use.

Growth factor assay. Growth enhancement of *Campylobacter* species by siderophores on the two media was determined by placing filter paper disks on plates inoculated with the test organism to yield isolated colonies at a concentration slightly less than that for confluent growth. Growth around the disks was observed after 24 h of incubation at 42°C under microaerobic conditions (5% O_2, 10% CO_2, 85% N_2).

RESULTS

All the *Campylobacter* strains grew well on the GC base medium. Growth inhibition was noted when disks were impregnated with 500 μg of human transferrin (Sigma Chemical Co., St. Louis, Mo.) or with 320 μg of Desferal per disk.

Of the 20 *C. jejuni* strains tested on medium A, all utilized ferrichrome, 16 utilized enterobactin, but none utilized rhodotorulic acid (Table 1). All the *C. coli* strains grew well in the presence of enterobactin and ferrichrome, but none utilized the rhodotorulic acid. Of 10 *C. laridis* strains, 3 did not utilize enterobactin, whereas the ferrichrome enhanced the growth equally well for all the strains. None utilized rhodotorulic acid. Ten *C. jejuni* strains were tested on both media A and B with enterobactin and ferrichrome (Table 2). Three strains failed to utilize enterobactin on either medium A or B. The organisms did not utilize the enterobactin as well on medium B as they did on medium A, and only scant growth was noted on medium B with ferrichrome.

The size of the colonies growing around the disks diminished in relation to the availability of the diffused chelators

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TABLE 1. Growth response of *Campylobacter* species to exogenous source of siderophores

Approx diam (mm) of growth by species	No. of strains utilizing:		
	Enterobactin	Ferrichrome	Rhodotorulic acid
C. jejuni			
35–40	9	7	0
25–30	5	13	0
8–10	2	0	0
NG[a]	4	0	20
C. coli			
35–40	9	5	0
25–30	1	5	0
8–10	0	0	0
NG	0	0	10
C. laridis			
35–40	0	10	0
25–30	5	0	0
8–10	2	0	0
NG	3	0	10

[a] NG, No growth.

(Fig. 1). Similar results were observed with disks impregnated with 20 μl of 0.1% each of ferric ammonium citrate, ferrous sulfate, and iron dextran when these were placed on the inoculated iron-deficient medium. The diameter of bacterial growth around the disk charged with iron or siderophores was used as a measure of the iron-acquiring effectiveness of the exposed bacterial strain.

DISCUSSION

The iron supplied in ferric ammonium citrate, ferrous sulfate, and iron dextran induced the growth of all the *Campylobacter* strains tested on media A and B. That no growth was observed without exogenous iron suggests that inhibition was due to the absence of free iron in the two media. The amount of free iron in medium B has been reported to be 8.0 ± 1.2 μM as measured by atomic absorption spectroscopy (8).

The growth of *Campylobacter* species on media A and B was also restored by an exogenous supply of siderophores (Fig. 1). This growth ranged from large, nearly confluent colonies around the disk where chelator concentrations were

TABLE 2. Evaluation of iron-binding capacities of media A and B

C. jejuni strain	Approx diam (mm) of siderophore-induced growth in:			
	Medium A		Medium B	
	Enterobactin	Ferrichrome	Enterobactin	Ferrichrome
B1	35–40	25–30	8–10	8–10
B2	35–40	35–40	25–30	8–10
B3	NG[a]	25–30	NG	8–10
B4	NG	25–30	NG	8–10
B5	35–40	35–40	25–30	8–10
B6	35–40	25–30	25–30	8–10
B7	35–40	25–30	25–30	8–10
B8	NG	25–30	NG	8–10
B9	35–40	25–30	25–30	8–10
B10	35–40	25–30	35–40	8–10

[a] NG, No growth.

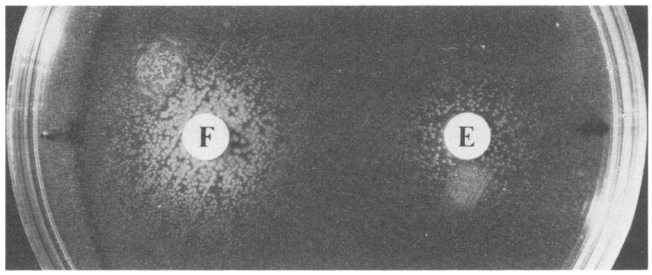

FIG. 1. Growth of *C. jejuni* on medium A around disks charged with ferrichrome (F) and enterobactin (E).

maximum to small colonies as chelators were diluted by diffusion through the medium. These data indicate that *Campylobacter* strains are not able to produce siderophores which can compete with transferrin or Desferal for iron. The data also suggest that most (82%) of the *Campylobacter* species tested have the membrane receptors for enterobactin and ferrichrome, a phenomenon also observed in strains of *S. typhimurium* (7) and *S. flexneri* (16).

Campylobacter strains appear to differ from *Yersinia enterocolitica* strains, which readily utilize iron complexed with Desferal. In a mouse lethality assay of *Yersinia* species, Desferal lowered the 50% lethal dose more than 100,000-fold (20). In contrast, we found that *C. jejuni* strains generally have a 50% lethal dose of 1.6×10^9 organisms, but pretreatment of mice with 5 mg of Desferal completely protected the mice. Preliminary results from pretreatment of mice with free iron indicate that the 50% lethal dose is actually lowered. These observations were also reported for *Listeria monocytogenes* (22) and are thought to reflect a depletion of iron in the mouse body by the formation of Desferal-iron complexes which the bacterial strain can not use.

These data and previous publications suggest that *Campylobacter* species have acquired the ability to scavenge siderophores produced by other organisms. In the host intestine, which is a heterogeneous competitive environment, *Campylobacter* strains may with other organisms cause synergistic infections. More research is required to actually demonstrate this potential pathogenicity determinant.

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