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Naturally Occurring Auxotrophs of *Campylobacter jejuni* and *Campylobacter coli*

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The nutritional requirements for 439 *Campylobacter jejuni* isolates and 46 *Campylobacter coli* isolates were determined by using a previously described chemically defined medium, campylobacter defined medium. With this medium, 45% of both human and nonhuman *C. jejuni* isolates demonstrated auxotrophic requirements. None of the 46 *C. coli* isolates studied demonstrated requirements for amino acids on campylobacter defined medium. The most common auxotrophic requirement among *C. jejuni* isolates was for methionine, which was present as a single requirement or in combination with other markers in 21% of human and 28% of nonhuman isolates. There was no correlation between plasmid carriage and auxotype, and a comparison of the Lior serotypes of 472 of the strains showed a correlation only between proline auxotrophs and Lior serotype 11 for strains isolated in the Seattle-King County region.

In our previous studies, we described a chemically defined medium that supported the growth of both *Campylobacter jejuni* and *Campylobacter coli* (10), which are common causes of acute enteritis among humans and animals (2). Using this medium, designated campylobacter defined medium (CDM), we demonstrated that 58% of the *C. jejuni* isolates required at least one amino acid for growth, while all of the *C. coli* isolates were prototrophic for the markers tested (10). The auxotyping system was also shown to be useful for identifying related organisms in three small outbreaks of campylobacteriosis.

In the present study, we sought to determine the presence of auxotrophic mutations in a larger sample of 485 campylobacter isolates and to determine whether correlations among auxotype, plasmid carriage, or Lior serotype could be noted.

MATERIALS AND METHODS

Bacterial strains. Strains of campylobacter were obtained from the following sources and laboratories: 363 *C. jejuni* isolates and 38 *C. coli* isolates from C. Nolan, Seattle-King County Department of Public Health, Seattle, Wash.; 39 *C. jejuni* isolates and 8 *C. coli* isolates from M. Blaser, Veterans Administration Medical Center, Denver, Colo.; 31 *C. jejuni* isolates from C. Fennell, Harborview Medical Center, Seattle, Wash.; and 6 *C. jejuni* isolates from the Centers for Disease Control, Atlanta, Ga. The isolates from the Department of Public Health were a subset of the strains from a Food and Drug Administration project to study the flow of campylobacter from food products of animal origin to humans (4, 5, 11). Isolates were chosen so as to represent a wide diversity of serotypes and plasmid profiles. The isolates from M. Blaser were human strains collected from laboratories around the United States. Some of these strains were described previously (1). All isolates obtained from C. Fennell were rectal isolates from homosexual males. The isolates from the Centers for Disease Control were Lior serotype 11 and were obtained from human cases of campylobacteriosis occurring in four different states (Illinois, two isolates; Georgia, two isolates; Michigan and Massachusetts, one isolate each). None of the 80 previously described campylobacter isolates (10) were included in this analysis.

A total of 279 isolates (254 *C. jejuni* and 25 *C. coli*) were of human origin, and 205 isolates (184 *C. jejuni* and 21 *C. coli*) were obtained from chickens at a local poultry processor. One isolate (*C. jejuni*) was obtained from a dog with diarrhea. All isolates were initially identified by using established criteria (8). In addition, a sample of 52 hippurate-positive organisms and all hippurate-negative organisms were identified to the level of species by using a whole-cell DNA:DNA homology test (12). Of the hippurate-negative organisms, 15 were classified as *C. jejuni* on the basis of this genetic test; the remaining 46 were identified as *C. coli*. All hippurate-positive organisms were confirmed as *C. jejuni*.

Auxotyping and serotyping. Auxotyping was performed by using CDM as previously described (10). Briefly, CDM is a chemically defined medium consisting of five solutions of salts and amino acids (grouped to facilitate preparation) and 12 individual components. Complete CDM consists of agar containing all solutions and components, while the typing media include a series of agar plates of CDM each deficient in one or more nutrients. Organisms were harvested after 18 to 24 h of growth from Columbia blood agar plates (Prepared Media Laboratories, Tualatin, Oreg.), suspended in 0.1 M phosphate-buffered saline, pH 7.2, and inoculated onto plates by using a Steers replicator. Strains were tested for their ability to grow in the absence of proline, methionine, arginine, the combination of half-cystine and cysteine, and the combination of isoleucine, leucine, and valine. All isolates were tested three times. Isolates demonstrating growth on all of the typing media were designated as prototrophs by using the convention of Knapp and Holmes (6). Serotyping (Lior method for heat-labile antigens and Penner method for heat-stable antigens) was performed as previously described (9, 10). All isolates were examined for plasmids by using an alkaline sodium dodecyl sulfate method as previously described (11).

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TABLE 1. Distribution of auxotypes in campylobacter isolates

Auxotype[a] or growth	No. of strains	
	Human	Nonhuman
C. jejuni		
Arg^-	9	2
Arg^-, ILV^-	0	2
Arg^-, Met^-	0	2
CC^-	9	4
CC^-, Met^-	1	0
ILV^-	1	2
ILV^-, Met^-	4	6
Met^-	48	44
Met^-, Pro^-	1	0
Pro^-	5	9
Weak on complete CDM	7	0
Weak, one or two markers	24	12
Prototrophic	145	102
C. coli prototrophic	25	21

[a] Arg^-, Arginine requiring; Met^-, methionine requiring; Pro^-, proline requiring; ILV^-, isoleucine, leucine, and valine requiring; CC^-, half-cystine–cysteine requiring. Prototrophic, No amino acid requirements noted on CDM.

RESULTS

Distribution of auxotypes. A total of 439 *C. jejuni* isolates, including 15 hippurate-negative organisms, and 46 *C. coli* isolates were tested for nutritional requirements by using CDM. Upon testing, 43% of the human *C. jejuni* isolates and 45% of the nonhuman (poultry and dog) *C. jejuni* isolates demonstrated auxotrophic requirements. Of the 15 hippurate-negative *C. jejuni* isolates, 14 were prototrophic; that is, they did not demonstrate auxotrophic requirements for the markers tested. The remaining isolate required isoleucine, leucine, valine, and methionine for growth on CDM. None of the *C. coli* isolates demonstrated requirements for amino acids. The distribution of auxotypes for *C. jejuni* isolates is shown in Table 1. The relative distribution of auxotypes for *C. jejuni* isolates is shown in Fig. 1.

Of the *C. jejuni* isolates tested, 43 consistently produced scant but discernible growth on at least one of the typing media. These organisms did not meet the criteria for positive growth (>10 colonies or confluent growth within the inoculum area) but consistently showed 2 to 7 colonies 0.5 to 1 mm in diameter. The weak-growth phenotype was not associated with any particular auxotype (arginine, half-cystine–cysteine, methionine, isoleucine-leucine-valine, or proline), site of isolation of the strain (stool versus extraintestinal), or source of isolation (human versus nonhuman) (data not shown). Of the 43 isolates, 7 consistently produced weak growth on the complete CDM plate as well. When subcultured on fresh complete CDM plates, the organisms continued to show poor growth. However, these isolates grew well when subcultured on blood agar. The 43 isolates expressed 16 different Lior serotypes and thus did not represent a single strain. The addition of several other nutrients, such as sodium lactate, sodium acetate, and glycerin, to complete CDM failed to support more luxuriant growth of these isolates.

Correlation among auxotype, serotype, and plasmid carriage. A total of 472 of the campylobacter isolates were serotyped by the method of Lior. Of the 413 *C. jejuni* isolates tested, 57 were nontypable and 9 were rough. The six most common Lior serotypes were Lior 36 (82 isolates), Lior 4 (38 isolates), Lior 6 (27 isolates), Lior 2 (26 isolates), Lior 1 (24 isolates), and Lior 11 (23 isolates). Of the 14 *C. jejuni* isolates requiring proline, 11 were isolated in the Seattle-King County region and were Lior 11. Of these 11 proline-requiring strains, 9 were isolated from poultry and were devoid of plasmids. Six different Penner serotype profiles were observed among these nine isolates, however, indicating that they were not the same strain (data not shown). The remaining proline-requiring isolates were identified as Lior 9 and 1,24. Of six *C. jejuni* isolates from the Centers for Disease Control carrying the Lior 11 antigen, four were prototrophic, while two required methionine or both methionine and proline.

Among the 46 *C. coli* isolates tested by the Lior system, 23 serotypes were noted. Six isolates proved nontypable. The most common Lior serotype among the *C. coli* isolates was Lior 21 (five isolates). The remainder were a variety of serotypes.

Of the 485 isolates, 204 harbored plasmids; 36 carried two or more plasmids. No correlation could be established between the presence of a specific plasmid in an isolate and its nutritional requirements.

DISCUSSION

This study confirms our earlier observations that approximately one-half of *C. jejuni* isolates require at least one amino acid for growth when tested on chemically defined media. *C. coli* isolates, on the other hand, are normally prototrophic for the markers tested.

The relatively large number of strains consistently exhibiting weak growth on CDM is intriguing. A total of 43 (9%) of the isolates consistently showed two to seven colonies on one or more of the CDM plates. The majority of these isolates grew well on the remaining plates, including the complete defined medium, indicating that the genetic defect was specific to a single requirement and did not represent a generalized membrane permeability phenomenon. The possibility of selective carry-over of nutrients from initial passage on blood agar should be negligible due to the saline suspension step prior to inoculation onto CDM. Seven organisms, however, did show weak growth on all CDM plates while showing luxuriant growth on blood agar. This phenomenon was not observed in our earlier study (10),

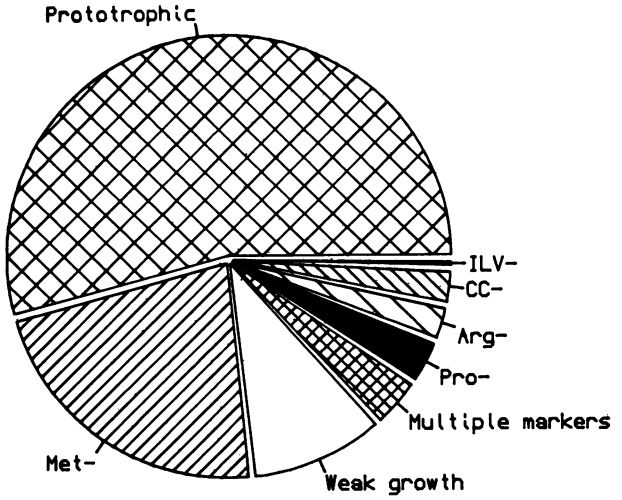

FIG. 1. Relative distribution of auxotypes of *C. jejuni* isolates as determined on CDM.

although on the basis of the studies of gonococcal auxotypes by Carifo and Catlin (3), it should not be unexpected. Such reactions suggest either that an additional cofactor or nutrient is required or is in limited supply in the medium or that an enzyme involved in a key metabolic pathway may not be fully functional. Although a variety of other amino acids and cofactors have been investigated, we have yet to identify a specific nutrient that produces more luxuriant growth of these strains.

It was interesting that the majority of proline-requiring isolates from the Seattle-King County area were Lior serotype 11. These 11 strains, however, represent only 50% of all Lior serotype 11 isolates noted in this study. The remaining serotype 11 isolates, including six human isolates from four different geographical regions of the United States, proved to be prototrophic or auxotrophic for other amino acids besides proline. Further examination of the Seattle serotype 11 isolates by using the Penner method demonstrated a variety of serotypes, suggesting that they are different strains. The association between the requirement for proline and Lior serotype 11 appears to be regional, much as the AHU auxotype (arginine, hypoxanthine, and uracil) of *Neisseria gonorrhoeae*, associated with disseminated gonococcal infections, proved to be unique to the Pacific Northwest (6, 7).

Recently, Blaser et al. (1) noted that *C. jejuni* isolates from cerebrospinal fluid are more likely to harbor auxotrophic mutations than are isolates from other extraintestinal sites. Unfortunately, clinical data on the human strains examined in this study (with the exception of those provided by M. Blaser) are not readily available, so that further correlations between auxotype and severity of disease or type of diarrhea are not currently possible.

Auxotyping appears to have limited utility as a strain-typing system, although it has proven to be of value in defining local outbreaks, particularly when serotyping data are not readily available. Of special importance, however, are the strains with multiple auxotrophic mutations and the large number of methionine auxotrophs, which we assume represent more than one genetic defect. These will be of value for genetic studies of the genus *Campylobacter*, especially for initiating construction of a genetic map of the chromosome of this organism. Auxotrophic mutants are also well suited for chromosomal mobilization studies. Complementation studies of genes involved in the metabolic pathways of proline and methionine synthesis are currently under way.

In summary, auxotrophic mutations appear to be relatively common in *C. jejuni* isolates but have not been observed in *C. coli* isolates. Auxotrophic markers have proven useful for limited epidemiologic studies but are perhaps more important as genetic markers for establishing a chromosomal map of this organism.

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