JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1987, p. 1560–1561
0095-1137/87/081560-02$02.00/0

Vol. 25, No. 8

Hydrolysis of Indoxyl Acetate by *Campylobacter* Species

CHARLES K. MILLS* AND ROBERT L. GHERNA
American Type Culture Collection, Rockville, Maryland 20852

Received 9 January 1987/Accepted 11 May 1987

One hundred and twelve *Campylobacter* strains comprising 15 species and subspecies were examined for their ability to hydrolyze indoxyl acetate. All strains of *C. coli*, *C. cryaerophila*, *C. fennelliae*, and *C. jejuni* hydrolyzed the compound, whereas three strains of *C. cinaedi* were negative and a fourth was weakly positive. Representatives of all other species were negative. Organisms that hydrolyzed indoxyl acetate did so regardless of the medium used.

Members of the genus *Campylobacter* are found in humans, other mammals, fowl, and plants, and some are associated with the disease state (1–4). Because of their implication in disease and epidemiological considerations, many of the *Campylobacter* research efforts have focused on devising tests for the identification of species. This has resulted in a number of physiological and biochemical tests that efficiently differentiate the species (5). However, the availability of additional tests which enable the rapid identification of *Campylobacter* species would be helpful. For a number of years, we have examined a variety of substrates that may be metabolized by *Campylobacter* species. Data are presented on hydrolysis of one of these compounds, indoxyl acetate by a bacterial esterase, which can be used in the rapid identification of *Campylobacter* species.

The microorganisms used in this study were kindly provided by C. M. Patton of the Centers for Disease Control, Atlanta, Ga.; J. Bryner of the National Animal Disease Center, Ames, Iowa; C. Fennell of Harborview Medical Center, Seattle, Wash.; M. Hines of the University of Maryland, College Park; P. J. Rothenberg of the U.S. Department of Agriculture, Beltsville, Md.; and the American Type Culture Collection, Rockville, Md.

A variety of media was used to propagate the organisms during the testing of hydrolysis of indoxyl acetate. This was done to ascertain whether the type used affected the outcome of the tests. *Campylobacter coli* and *C. jejuni* were grown on plates of Campylobacter Agar (BBL Microbiology Systems, Cockeysville, Md.), Trypticase soy agar (BBL) with 5% defibrinated sheep blood, and Brucella Albimi agar (GIBCO Diagnostics, Madison, Wis.). *Campylobacter cryaerophila*, "*C. fecalis*," *C. fetus* subsp. *fetus*, *C. fetus* subsp. *venerealis*, *C. hyointestinalis*, *C. laridis*, *C. pylori*, and *C. sputorum* subsp. *bubulus* were grown on plates of Trypticase soy agar with 5% defibrinated sheep blood and Brucella Albimi agar. *Campylobacter cinaedi* and *C. fennelliae* were propagated on plates of Brucella Albimi agar with 10% sheep blood. All were incubated at 37°C in a GasPak jar (BBL) under a reduced oxygen atmosphere generated by use of a H_2 and CO_2 generator envelope (BBL) but no catalyst. *C. cinaedi* and *C. fennelliae* were incubated for 7 days, and all others were held for 48 h.

Campylobacter sputorum subsp. *mucosalis*, and *C. sputorum* subsp. *sputorum* were propagated on plates of Trypticase soy agar with 5% defibrinated sheep blood and Brucella Albimi agar anaerobically in a GasPak jar with catalysts and a H_2 and CO_2 generator envelope at 37°C for 48 h. *Campylobacter nitrofigilis* was grown on plates of Brucella Albimi agar with 1.5% NaCl (wt/vol) and incubated anaerobically in a GasPak jar at 25°C for 72 h.

Indoxyl acetate (Sigma Chemical Co., St. Louis, Mo.) disks were prepared by adding 50 μl of a 10% (wt/vol) solution of the compound in acetone to a concentration disk (0.25-in. [ca. 0.64-cm] diameter; Difco Laboratories, Detroit, Mich.) and allowed to air dry. After being dried, the disks were stored at 4°C in an amber bottle in the presence of silica gel.

Indoxyl acetate hydrolysis was detected by two methods. In one method, colonies of a *Campylobacter* species were placed on a disk and a drop of sterile distilled water was

TABLE 1. Indoxyl acetate hydrolysis by *Campylobacter* species

Organism	Origin[a]	No. of strains	No. of strains hydrolyzing indoxyl acetate
C. cinaedi	HMC	4	1 (weak)
C. coli	ATCC	5	5
	CDC	5	5
C. cryaerophila	ATCC	2	2
"*C. fecalis*"	ATCC	3	0
C. fennelliae	HMC	3	3
C. fetus subsp. *fetus*	ATCC	8	0
	CDC	6	0
C. fetus subsp. *venerealis*	ATCC	2	0
	CDC	2	0
	NADC	8	0
C. hyointestinalis	ATCC	1	0
C. jejuni	ATCC	17	17
	CDC	10	10
	USDA	15	15
	U.Md.	8	8
C. laridis	ATCC	3	0
C. nitrofigilis	ATCC	1	0
C. pylori	ATCC	2	0
C. sputorum subsp. *bubulus*	ATCC	2	0
C. sputorum subsp. *mucosalis*	ATCC	2	0
C. sputorum subsp. *sputorum*	ATCC	1	0
	CDC	2	0

[a] HMC, Harborview Medical Center; ATCC, American Type Culture Collection; CDC, Centers for Disease Control; NADC, National Animal Disease Center; USDA, U.S. Department of Agriculture; U.Md., University of Maryland.

* Corresponding author.

added. If indoxyl acetate was hydrolyzed, a color change to dark blue occurred in 5 to 10 min. No color change indicated hydrolysis had not taken place. In the alternative method, colonies were suspended in 0.3 ml of sterile distilled water. The disk was added, and, if positive, a color change to blue occurred in 10 to 15 min at room temperature. All strains of *C. coli*, *C. cryaerophila*, *C. fennelliae*, and *C. jejuni* hydrolyzed indoxyl acetate, whereas three of the four strains of *C. cinaedi* tested gave negative reactions (Table 1). One strain of *C. cinaedi* gave a weak reaction (Table 1). All the other organisms were negative. All strains that hydrolyzed indoxyl acetate did so regardless of the culture medium used. All reactions were distinct, and the shelf life of the disks was in excess of 1 year if protected from light and under a desiccant at 4°C.

In summary, the utility of the test lies in the fact that it is rapid and selective for a limited number of *Campylobacter* species. Its low expense also makes it cost-effective for routine laboratory use and beneficial for those interested in *Campylobacter* identification and taxonomy.

We thank B. S. Roberson of the Department of Microbiology, University of Maryland, for a critical review of the manuscript. We also thank Ellen Baque for typing the manuscript.

This investigation was supported in part by grant BSR 8415014 from the National Science Foundation.

LITERATURE CITED

1. **Blaser, M. J., J. Cravens, B. W. Powers, and W. L. Wang.** 1978. *Campylobacter* enteritis associated with canine infections. Lancet **ii**:979–981.
2. **McClung, C. R., D. G. Patriquin, and R. E. Davis.** 1983. *Campylobacter nitrofigilis* sp. nov., a nitrogen-fixing bacterium associated with roots of *Spartina alterniflora* Loisel. Int. J. Syst. Bacteriol. **33**:605–612.
3. **Skirrow, M. B.** 1977. *Campylobacter* enteritis, a "new" disease. Br. Med. J. **2**:9–11.
4. **Smibert, R. M.** 1969. *Vibrio fetus* var. *intestinalis* isolated from intestinal contents of birds. Am. J. Vet. Res. **30**:1437–1442.
5. **Smibert, R. M.** 1984. Genus *Campylobacter* Sebald and Véron 1963, 907^{AL}, p. 111–118. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.