APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1987, p. 375–378
0099-2240/87/020375-04$02.00/0
Copyright © 1987, American Society for Microbiology
Vol. 53, No. 2

Susceptibility of *Campylobacter jejuni* and *Yersinia enterocolitica* to UV Radiation

RICHARD C. BUTLER,[1] VIDAR LUND,[2] AND DALE A. CARLSON[1]*

Environmental Engineering and Science Program, Civil Engineering Department, University of Washington, Seattle, Washington 98195,[1] and Department of Water Hygiene, National Institute for Public Health, 0460 Oslo 4, Norway[2]

Received 25 August 1986/Accepted 5 November 1986

Two enteric pathogens, *Campylobacter jejuni* and *Yersinia enterocolitica* serogroup O:3, together with *Escherichia coli*, were investigated for susceptibility to UV radiation at 254 nm. The UV dose required for a 3-log reduction (99.9% inactivation) of *C. jejuni*, *Y. enterocolitica*, and *E. coli* was 1.8, 2.7, and 5.0 mWs/cm^2, respectively. Using *E. coli* as the basis for comparison, it appears that *C. jejuni* and *Y. enterocolitica* serogroup O:3 are more sensitive to UV than many of the pathogens associated with waterborne disease outbreaks and can be easily inactivated in most commercially available UV reactors. No association was found between the sensitivity of *Y. enterocolitica* to UV and the presence of a 40- to 50-megadalton virulence plasmid.

Campylobacter jejuni and *Yersinia enterocolitica* are widely recognized as important etiological agents of gastroenteritis all over the world. Whereas one to two decades ago these organisms were seldom associated with bacterial diarrhea, today they are isolated at frequencies which are commonly reported for the more familiar gastrointestinal pathogens—*Salmonella* and *Shigella* species (4, 5, 28). In fact, in some surveys, *Campylobacter enteritis* has emerged as the most frequently reported cause of acute bacterial diarrhea (4, 28).

C. jejuni and *Y. enterocolitica* are commonly found in nature and have been isolated from a variety of domestic and wild birds and animals (17), which may constitute the main reservoirs for these microorganisms (3). Most of these animals are otherwise healthy carriers usually associated with water habitats. Although drinking fecally contaminated water may not be the primary source of human infections, both microorganisms have been implicated in several waterborne disease outbreaks (7, 10, 22, 24, 29, 30). Considering the ability of *Y. enterocolitica* to grow in waters at temperatures as low as 4°C (13) and the ability of *C. jejuni* to survive for several weeks in low-temperature water (11) as well as its low infectious dose (26, 28), the potential for waterborne outbreaks of pathogens of these two genera can be appreciated.

Many drinking water systems in Norway are unprotected surface waters receiving little or no treatment. Waterborne outbreaks of yersiniosis and campylobacteriosis are often associated with systems exhibiting these features. Indeed, Norway has experienced outbreaks of both yersiniosis and campylobacteriosis where drinking water was suspected as the source of infection (20; B. Godronsen, K. Melby, S. Gregusson, and O. P. Dahl, III International Workshop on *Campylobacter* Infections in Canada, Montreal, 1985). Concern for the safety of its water supplies prompted the National Institute of Public Health in Oslo, Norway, to determine the susceptibility of *C. jejuni* and *Y. enterocolitica* to the principal drinking water disinfectants currently in use in Norway—chlorine and UV radiation.

In this article we present the results of a laboratory bench-scale study of the capacity of UV radiation to inactivate *C. jejuni* and *Y. enterocolitica*. Strains commonly found in Norway and Europe (15) were used as test organisms. Because UV technology is traditionally designed and operated on a coliform basis, *E. coli* sensitivity was also determined. This also provided a basis for comparison with other UV-bacteria studies. Differences in the sensitivity of a virulent and an avirulent *Y. enterocolitica* strain were examined.

MATERIALS AND METHODS

Test organisms. *C. jejuni* biotype 1 strain 709/84 and *Y. enterocolitica* serogroup O:3 biotype 4 strain 304/84 were obtained from J. Lassen (National Institute of Public Health, Oslo). Virulent (P^+) and avirulent (P^-) *Y. enterocolitica* serogroup O:3 biotype 4 strain 1526/81 isolates were obtained from G. Kapperud (Norwegian Defence Microbiological Laboratory, Oslo). *Y. enterocolitica* strain 1526/81 P^+ is a wild-type strain which harbors a 40- to 50-megadalton (MDa) virulence plasmid. *Y. enterocolitica* strain 1526/81 P^- is an isogenic mutant which has spontaneously lost plasmid DNA. All of the *Y. enterocolitica* and *C. jejuni* strains are human clinical isolates. *E. coli* was isolated from the raw water supply for Eidsberg, Norway. All test organisms were stored at −80°C in Greave solution.

Growth media and growth conditions. Procedures for the preparation of *Y. enterocolitica* 304 and *E. coli* suspensions for UV inactivation were identical except for the time and temperature of incubation, which were 48 h at 22°C for *Y. enterocolitica* and 24 h at 37°C for *E. coli*. Suspensions were prepared by transferring frozen bacterial strains onto horse blood-agar plates. Single colonies from incubated plates were inoculated into lactose-peptone broth and incubated under the appropriate conditions. Bacterial suspensions were harvested by centrifugation at 22,500 × *g* for 10 min (Sorval ultracentrifuge). Pelleted bacteria were suspended in 0.1% peptone water. Centrifugation and suspension were repeated three times to facilitate complete removal of the growth medium. The bacterial density was adjusted to approximately 10^8 CFU/ml with sterile, distilled water and stored at 4°C. This stock suspension was subsequently diluted with distilled water to the desired concentration for the irradiation experiments. Stock solutions were discarded after 7 days.

Y. enterocolitica 1526/81 P^+ and P^- suspensions were prepared in the same manner as for *Y. enterocolitica* strain

* Corresponding author.

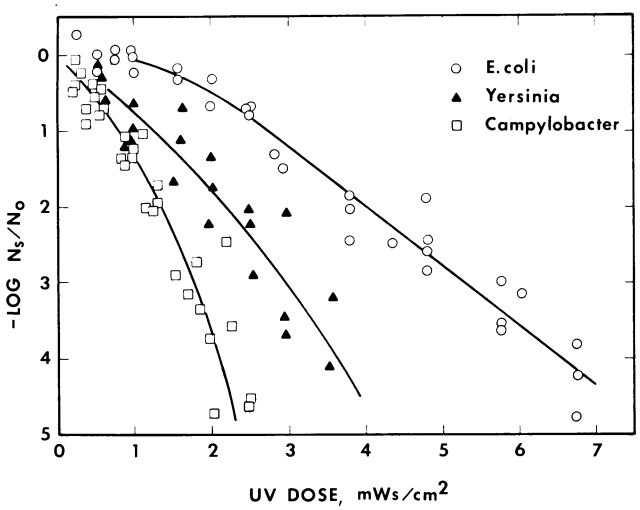

FIG. 1. UV inactivation curves of *C. jejuni*, *Y. enterocolitica* 304, and *E. coli*. Initial concentrations were approximately 10^6 to 10^7 CFU/ml.

304, except that single colonies were inoculated into Eagle basal medium with 10% fetal calf serum at 37°C rather than into lactose-peptone broth at 37°C. This methodology allowed the virulence plasmid surface factors of the virulent strain 1526/81 P^+ to evolve (autoagglutination) (18).

Frozen *C. jejuni* cells were inoculated on fresh horse blood-agar plates. Inoculated plates were incubated at 42°C for 48 h in a microaerophilic atmosphere containing 5% O_2, 10% CO_2, and 85% N_2. One colony from an incubated plate was transferred to Preston broth and incubated as noted above. The 48-h incubation period for Preston broth was strictly observed because *C. jejuni* tends to aggregate at high cell densities, thereby making it difficult to obtain a well-dispersed suspension with single cells. The mature bacterial suspension was subsequently prepared for irradiation experiments by dilution with sterile distilled water. Separation of *C. jejuni* from Preston broth by centrifugation was not practical because of our inability to obtain a dispersed suspension of *C. jejuni* after resuspension. Stock solutions were discarded after 1 day.

Irradiation procedure. The batch-irradiation apparatus and experimental methodology were fashioned after those of Qualls and Johnson (25). A collimated beam of UV light (254 nm) was directed at a mixed, thin-layer, bacterial suspension of approximately 10^6 to 10^7 CFU/ml. The suspension was irradiated in a small petri dish (60-mm diameter) which provided an initial liquid depth of 1.0 cm. Various UV doses were surveyed during an irradiation experiment by withdrawing samples at different exposure times. A self-imposed minimal depth of 0.8 cm controlled the total number of samples per experiment. To avoid photoreactivation, withdrawn samples were immediately suspended in 0.1% peptone water and enumerated by surface spreading or membrane filtration in triplicate followed by incubation in the dark. *Y. enterocolitica* and *E. coli* were enumerated on fresh, horse blood-agar plates at 37°C for 18 to 24 h. *C. jejuni* was enumerated on predried, horse blood-agar (dried at 37°C for 1 h and overnight at room temperature) at 42°C for 48 h in a microaerophilic atmosphere. Controls were obtained for all experiments by subjecting a suspension to all experimental conditions except UV exposure. All experiments were performed at room temperature.

Incident UV intensity at the liquid surface (I_0) was measured at 254 nm with a J-260 digital radiometer with a J-260-LA sensor ASSY-254 nm detector (Ultra-Violet Products, Inc., San Gabriel, Calif.). Sample absorbance at 254 nm was measured in a 1-cm quartz cuvette with a Hitachi Perkin-Elmer double-beam spectrophotometer. The average reaction rate intensity (I_{avg}), which was used to calculate UV dose, was defined by the following equation (25):

$$I_{avg} = 2\, I_0 r_0\, [1 - 10^{-A(r - r_0)}]/2.303\, A(r^2 - r_0^2)$$

where r_0 is the light distance (centimeters) from the center of the UV lamp to the liquid surface, r is r_0 plus the depth of liquid, and A is the absorbance per centimeter at 254 nm (base 10). Because of the sampling procedure used, incident surface intensity decreased with each sample withdrawal. However, the calculated variation in surface intensity was so small (ca. 1% based on an initial r_0 of 30.2 cm) that the initial surface intensity was used for all dose calculations. Similarly, when absorbance was minimal (<0.05/cm), UV dose was calculated by using only I_0 and the exposure time. The dose (milliwatt-seconds per square centimeter; alternate units are watt-seconds per square meter) was calculated as a cross-product of intensity (milliwatts per square centimeter) and exposure time (seconds). Inactivation data were plotted as log N_s/N_0 versus UV dose, where N_s is the concentration of surviving microorganisms and N_0 is the initial microorganism concentration (14).

RESULTS AND DISCUSSION

UV inactivation results for *C. jejuni*, *Y. enterocolitica*, and *E. coli* are presented in Fig. 1. Each point represents one irradiation experiment, and the lines are subjective interpretations.

The results display slight but noticeable differences in the UV sensitivity of the three bacteria. *C. jejuni* was the most sensitive of the bacteria, followed in decreasing sensitivity by *Y. enterocolitica* and *E. coli*. Both *C. jejuni* and *Y. enterocolitica* commenced inactivation with the initial application of UV. *E. coli*, on the other hand, exhibited an initial resistance to inactivation requiring nearly 1 mWs/cm^2 before inactivation occurred. None of the inactivation curves displayed a flattening of the curve at higher UV doses, a phenomenon characteristic of a resistant subpopulation.

A parameter used for comparing the bacterial sensitivity of different disinfection studies is the 3-log (99.9%) inactivation dose. For this study, the 3-log doses of *C. jejuni*, *Y. enterocolitica*, and *E. coli* were 1.8, 2.7, and 5.0 mWs/cm^2, respectively. These doses are in reasonable agreement with doses reported for other vegetative bacteria. For example, Chang et al. (8) found that *E. coli*, *Salmonella typhi*, *Shigella sonnei*, and *Staphylococcus aureus* exhibited a 3-log dose (7 mWs/cm^2) comparable to our strain of *E. coli*. Carlson et al. (6), using a slightly different irradiation apparatus, described similar 3-log doses (7 to 10 mWs/cm^2) for *E. coli* and an avirulent *Y. enterocolitica* strain. Additionally, an investigation of *Legionella pneumophila* reported a susceptibility to UV similar to that of *Y. enterocolitica* 304 in this experiment (1). Only a few recent UV studies have described markedly different results: the *E. coli* strain (3-log dose of 14 mWs/cm^2) used by Severin et al. (27) and the virulent *Y. enterocolitica* strain, serogroup O:8 (3-log dose of 22 mWs/cm^2), studied by Carlson et al. (6). No previous reference to UV and *C. jejuni* was found.

It has been suggested that plasmids, particularly plasmids associated with virulence, may be associated with providing

enhanced disinfection resistance to microorganisms (9). This possible association was studied by determining the difference in the sensitivity of the 42-MDa plasmid-containing isolates and the plasmid-cured isolates of *Y. enterocolitica* 1526/81. Previous studies have already demonstrated an indirect correlation between a 40- to 48-MDa plasmid and the virulence of *Y. enterocolitica* 1526/81 (2, 18). Inactivation results gave little indication that the virulence plasmid enhanced the resistance of *Y. enterocolitica* 1526/81 to UV. This is in contrast to the results of Carlson et al. (6), who found that two avirulent strains were considerably more sensitive to UV than a virulent (plasmid-containing) *Y. enterocolitica* strain. In that study, the virulent *Y. enterocolitica* strain was a member of serogroup O:8 and contained two plasmids of 41 and 73 MDa, both of which were absent from the avirulent strains.

A study by Kay et al. (19) indicated the possibility that an 82-MDa plasmid could be associated with virulence in *Y. enterocolitica*. Because this plasmid was present in the strain tested by Carlson et al. and not in our virulent strain, there exists the possibility that the strong resistance exhibited by the virulent strain of Carlson et al. could be associated with the expression of virulence. However, the transconjugation experiments of Heeseman and Laufs (12) provided direct proof that virulence, as measured by mouse mortality, the same measure used by Kay et al., was associated with the 42-MDa plasmid.

Studies with disinfectants other than UV have provided little support for the concept that the virulence plasmid of *Y. enterocolitica* is associated with enhanced disinfection resistance (6, 16, 21). When a difference in the resistance of the virulent and avirulent strains was distinguishable in other studies, it was rather small, i.e., <1 log unit, and the virulent *Y. enterocolitica* strain was not always the more resistant strain (21; Gondrosen et al., III Workshop on *Campylobacter* Infections in Canada). These studies indicate that if the virulence plasmid does provide protection from disinfection, it is specific for bacterial strain, type of disinfectant, and environmental conditions (e.g., pH and temperature).

There was concern that the high cell densities used in the *E. coli* inactivation experiments were masking the true sensitivity of the microorganism. At high cell densities, cells may contribute significantly to the absorbance of a suspension through "shading," an effect described by Morowitz (23), and thus hinder inactivation as demonstrated by Jagger et al. at *E. coli* densities greater than 10^7 CFU/ml (15). High cell densities may also promote cell aggregation, which could produce a "shoulder" on the inactivation curve, i.e., initial resistance. Thus, an experiment was performed with *E. coli* at a cell density of 10^4 CFU/ml to evaluate the effect of lower cell concentrations on inactivation. A concentration of 10^4 CFU/ml was chosen because it more closely represents coliform concentrations found in nature, yet provides a suspension dense enough to obtain a reasonable range of data. The results, which indicate there is little difference in the initial resistance or the 3-log dose for *E. coli* suspensions at 10^7 and 10^4 CFU/ml, suggest that the initial resistance of *E. coli* is indeed an inherent cell characteristic.

In conclusion, the UV dose required to effectively inactivate *C. jejuni* biotype 7 and *Y. enterocolitica* serogroup O:3 is slightly lower than the doses reported for other vegetative bacteria. When compared with the range of pathogens that are of concern in drinking water, *Campylobacter* and *Yersinia* strains used in this study are two of the more sensitive to UV (8). The capacity of most commercially available UV reactors (30 mWs/cm^2) is more than adequate to effectively inactivate these bacteria, assuming that plug flow occurs in the reactor. *E. coli*, because of its greater resistance to UV, is an adequate, if not conservative, model for the inactivation of these pathogenic bacteria with UV.

Little difference was exhibited in the resistance of a 40- to 48-MDa virulence plasmid-containing *Y. enterocolitica* serogroup O:3 strain and its plasmid-cured mutant, thereby lending no support to the suggestion that virulence plasmids may be associated with enhanced disinfection resistance.

ACKNOWLEDGMENTS

This work was supported by the Royal Norwegian Council for Scientific and Industrial Research, Oslo, NTNF no. 1523.13191, and the Valle Scholarship and Scandinavian Exchange Program, University of Washington, Seattle.

We thank Hans Utkilen for his constructive suggestions, Turid Langholdt and Therese Hagtvedt for their unending assistance in the laboratory, Bobbie Greer for technical assistance with manuscript preparation, and James Staley for his review of the manuscripts.

LITERATURE CITED

1. **Antopol, S. C., and P. D. Ellner.** 1979. Susceptibility of *Legionella pneumophila* to ultraviolet radiation. Appl. Environ. Microbiol. **38**:347–348.
2. **Ben-Gurion, R., and A. Schafferman.** 1981. Essential virulence determinants of different *Yersinia* species are carried on a common plasmid. Plasmid **5**:183–187.
3. **Blaser, M. J., F. M. LaForce, N. A. Wilson, and W.-L. L. Wang.** 1980. Reservoirs for human campylobacteriosis. J. Infect. Dis. **141**:665–669.
4. **Blaser, M. J., J. G. Wells, R. A. Feldman, R. A. Pollard, J. R. Allen, and the Collaborative Diarrheal Disease Study Group.** 1983. *Campylobacter enteritis* in the United States. A multicenter study. Ann. Intern. Med. **98**:360–365.
5. **Bottone, E. J.** 1977. *Yersinia enterocolitica*: a panoramic view of a charismatic microorganism. Crit. Rev. Microbiol. **5**:211–241.
6. **Carlson, D. A., R. W. Seabloom, F. B. DeWalle, T. F. Wetzler, J. Engeset, R. Butler, S. Wangsuphachart, and S. Wang.** 1985. Ultraviolet disinfection of water for small water supplies. Publication no. EPA/600/52-85/092. U.S. Environmental Protection Agency, Water Engineering Research Laboratory, Cincinnati, Ohio.
7. **Centers for Disease Control.** 1982. Outbreak of *Yersinia enterocolitica*—Washington state. Morbid. Mortal. Weekly Rep. **31**: 562–564.
8. **Chang, J. C., S. F. Ossoff, D. C. Lobe, M. H. Dorfman, C. M. Dumais, R. G. Qualls, and J. D. Johnson.** 1985. UV inactivation of pathogenic and indicator microorganisms. Appl. Environ. Microbiol. **49**:1361–1365.
9. **Davies, J., and D. I. Smith.** 1978. Bacterial resistance to antimicrobial agents. Annu. Rev. Microbiol. **32**:496–518.
10. **Eden, K. V., M. L. Rosenberg, M. Stoopler, B. T. Wood, A. K. Highsmith, P. Skaliy, J. A. Wells, and J. C. Feeley.** 1977. Waterborne gastroentestinal illness at a ski resort. Public Health Rep. **92**:245–250.
11. **Gondrosen, B.** 1985. Survival of thermotolerant *Campylobacters* in water. Department of Food Hygiene, Norwegian College of Veterinary Medicine, Oslo.
12. **Heeseman, J., and R. Laufs.** 1983. Construction of a mobilizable *Yersinia enterocolitica* virulence plasmid. J. Bacteriol. **155**: 761–767.
13. **Highsmith, A. K., J. C. Feeley, P. S. Skaley, J. G. Wells, and B. T. Wood.** 1977. Isolation of *Yersinia enterocolitica* from well water and growth in distilled water. Appl. Environ. Microbiol. **34**:745–750.
14. **Jagger, J.** 1967. Introduction to research in UV photobiology. Prentice-Hall, Inc., Engelwood Cliffs, N.J.
15. **Jagger, J., T. Fossum, and S. McCall.** 1975. Ultraviolet irradiation of suspensions of micro-organisms: possible errors involved in the estimation of average fluence per cell. Photochem.

Photobiol. **21**:379–382.
16. **Jarakeh, M. S., J. D. Berg, J. C. Hoff, and A. Matin.** 1985. Susceptibility of chemostat-grown *Yersinia enterocolitica* and *Klebsiella pneumoniae* to chlorine dioxide. Appl. Environ. Microbiol. **49**:69–72.
17. **Kapperud, G.** 1983. *Yersinia enterocolitica* og *Campylobacter jejuni/coli* i naeringsmiddelhygienisk sammenheng. Internt notat for norges veterinaerhøgskole, Oslo.
18. **Kapperud, G., H.-J. Skarpeid, R. Solberg, and T. Bergan.** 1984. Outer membrane proteins and plasmids in different *Yersinia enterocolitica* isolated from man and animals. Acta Pathol. Microbiol. Immunol. Scand. Sect. B **93**:27–34.
19. **Kay, B. A., K. Wachsmuth, and P. Gemski.** 1982. New virulence-associated plasmid in *Yersinia enterocolitica*. J. Clin. Microbiol. **15**:1161–1163.
20. **Lassen, J.** 1972. *Yersinia enterocolitica* in drinking water. Scand. J. Infect. Dis. **4**:125–127.
21. **Lund, V.** 1985. Overlevelse av *Campylobacter*, *Yersinia* og *E. coli* in vann. Delrapport 1: *Yersinia enterocolitica* og *E. coli* i klorholding vann. Drikkevannsrapport 14, NTNFs utvalg for drikkevannsforskning, Oslo.
22. **Mentzing, L. O.** 1981. Waterborne outbreaks of *Campylobacter enteritis* in central Sweden. Lancet **ii**:352–354.
23. **Morowitz, H. J.** 1950. Absorption effects in volume irradiation of microorganisms. Science **111**:229–230.
24. **Palmer, S. R., P. R. Gally, and J. M. White.** 1983. Water-borne outbreak of *Campylobacter gastroenteritis*. Lancet **i**:287–290.
25. **Qualls, R. G., and J. D. Johnson.** 1983. Bioassay and dose measurement in UV disinfection. Appl. Environ. Microbiol. **45**: 872–877.
26. **Robinson, D. A.** 1981. Infective dose of *Campylobacter jejuni* in milk. Br. Med. J. **282**:1584.
27. **Severin, B. F., M. T. Suidan, and R. S. Engelbrecht.** 1983. Kinetic modeling of UV disinfection of water. Water Res. **17**:1669–1678.
28. **Skirrow, M. B.** 1982. *Campylobacter enteritis*—the first five years. J. Hyg. **89**:175–184.
29. **Taylor, D. N., M. Brown, and K. T. McDermott.** 1982. Waterborne transmission of *Campylobacter enteritis*. Microb. Ecol. **8**:347–354.
30. **Vogt, R. L., H. E. Sours, T. Barrett, R. A. Feldman, R. J. Dickinson, and L. Witherall.** 1982. *Campylobacter enteritis* associated with contaminated water. Ann. Intern. Med. **96**: 292–296.