APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1984, p. 378–380
0099-2240/84/020378-03$02.00/0

Vol. 47, No. 2

Trypsin-Treated Ma-104: A Sensitive Cell Line for Isolating Enteric Viruses from Environmental Samples

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Received 25 July 1983/Accepted 2 November 1983

During a 1-year survey of enteroviruses in wastewater samples from the Lorraine area, three widely used continuous monkey kidney cell lines were tested: BGM, Vero, and trypsin-treated Ma-104. Decontaminated samples from secondary wastewater treatment plants (influent or effluent) were directly inoculated onto cells, and viruses were revealed after two passages with a liquid medium technique. Out of the total percentage of positive isolates with the three systems (32.7) 24.7% were found with Ma-104, 14.1% with BGM, and only 1.7% with Vero cells. Poliovirus was recovered more frequently with Ma-104 (12.3%) than with BGM (1.7%). Reovirus (3.5%) and echovirus (1.7%) were only found with Ma-104 cells; however, BGM cells allowed the isolation of a few group B coxsackieviruses (5.9%). It must be pointed out that 7.0% of samples with an unconfirmed cytopathic effect were found with BGM against 3.4% found with Ma-104, but they did not have significant differences. Because of its large spectrum of sensitivity, easy maintenance, and resistance to toxic effects, trypsin-treated Ma-104 may be recommended in conjunction with other cell lines for the detection of viruses from environmental samples, especially with the use of a liquid method.

The wide diffusion of viruses in the environment is well known from many studies, but their amount varies greatly from one country to another (4, 7, 9, 13–15, 17, 20) and from one geographic area to another in the same country (1, 5, 10, 12). Furthermore, qualitative evaluation often shows that the viruses isolated are enteroviruses, some species of which, e.g., reoviruses, are rarely recovered, in contrast to polioviruses, coxsackieviruses, and echoviruses, which are most commonly isolated. Several reasons may be evoked to explain these results: (i) a limited number of cell lines are tested because of the toxic effects of the samples; (ii) cell sensitivity for the spectrum of propagated viruses varies (16, 19); (iii) isolation attempts with plaque assays or tube cultures with a fluid medium show that each has its own limitations (21, 22). All of these account for the restricted spectrum of viruses observed in the majority of studies, other than a few investigations (9, 17, 19), for which the cell systems tested are sometimes difficult to maintain routinely in the laboratory. This study reports the results from a 1-year survey of enteroviruses in wastewater samples from the Lorraine area, after direct inoculation onto three types of continuous monkey kidney cell lines.

MATERIALS AND METHODS

Samples examined. A total of 113 wastewater samples analyzed were taken from wastewater plants which used an activated sludge treatment method. The samples were half from raw sewage and half from secondary wastewater effluent. From 1 liter of water, 40 ml was treated by a method described elsewhere (3) and decontaminated with 1 ml of an antibiotic mixture ([per ml] 100,000 U of sodium penicillin, Sarbach Laboratories; 0.1 mg of streptomycin sulfate, Specia Laboratories; 0.05 mg of neomycin sulfate, Roussel-Uclaf Laboratories) and 1 ml of amphotericin B (5 μg/ml; Squibb Laboratories). After this treatment, the samples were stored at −20°C and then inoculated onto the cell cultures for about 3 days.

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Cell cultures. In this study, three monkey kidney cell lines were tested with a liquid medium technique: (i) BGM cells, an African green monkey kidney cell line obtained from Flow Laboratories, Inc., McLean, Va., were used between passages 74 and 85; (ii) Vero cells, an African green embryonic monkey kidney cell line normally maintained in our laboratory, were used between passages 89 and 112; (iii) Ma-104 cells, an African rhesus monkey kidney cell line, obtained from I.N.R.A., Plaisir Grignon, France, were used at passage levels of 60 to 92. BGM and Vero cells were cultivated in Eagle minimum essential medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 5% glutamine (Difco Laboratories, Detroit, Mich.) at 0.1%, $NaHCO_3$ at 1.12 mg/liter, 0.03 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), and 10% heat-inactivated calf serum (Seromed Laboratories) previously tested for the presence of neutralizing antibodies. The serum was replaced by 10% heat-inactivated fetal calf serum for Ma-104 cell cultures which grew well under these conditions.

Inoculation of cells. After elimination of the culture medium, 2 ml of each sample was inoculated in triplicate onto the confluent monolayer of each cell line (25-cm^2 culture flasks; Falcon Plastics, Oxnard, Calif.). At the same time, the Ma-104 cells were treated with 0.1 ml of trypsin at 100 μg/ml (Difco Laboratories). After incubation for 2 h at 37°C and elimination of inoculum, each bottle received 7 ml of Eagle minimum essential medium (GIBCO) with 2% calf serum or fetal calf serum. Cytopathic effects were read daily for 7 days, and two blind passages were performed.

Isolation and identification of viruses. For isolation, the plaque method was performed, using Eagle minimum essential medium with 2% fetal calf serum and 1% of a 1% solution of $MgCl_2$, 1.5% of a 1:1000 dilution of neutral red, and 1.8% of Difco agar as the overlay medium. All plaque-forming units obtained were inoculated onto BGM or Ma-104 cells grown to monolayers in plastic bottles. The viruses were identified with the microneutralization test by using flat-bottomed wells of tissue culture microtiter plates and the enterovirus immune serum pools of K. A. Lim and M. Benyesh-Melnick, obtained from National Institutes of

TABLE 1. Virus types recovered by various host cell systems from wastewater samples

Virus[a]	No. of positive specimens	No. of positive specimens from:			Total virus types
		Ma-104	BGM	Vero	
P 1	15	14	1	1	16
P 3	1	0	1	0	1
CB 2	2	2	1	0	3
CB 5	5	2	5	0	7
Echo 1	2	2	0	0	2
Reo 2	4	4	0	0	4
Unidentified	8	4	8	1	—

[a] P, Poliovirus; CB, coxsackievirus B; Echo, echovirus; Reo, reovirus.

Health, Bethesda, Md. Reoviruses were identified with hemalum eosin staining and typed by using the hemagglutination inhibition test against antisera.

RESULTS

Sample toxicity to cell cultures. The toxic effect of the samples varied among the three cell lines, but the difference was not significant ($P > 5\%$) (7% in Vero cells, 2% in BGM and Ma-104 cells). The use of culture medium containing 2% serum did not inhibit this toxicity.

Recovery of viruses from wastewater and distribution in various host cell systems. Of the 113 wastewater samples inoculated in parallel onto the three culture systems, 37 yielded virus-like cytopathic effects, i.e., 32.7%. Of these 37 positive samples, a single virus type was isolated from 24, two virus types were isolated from 5, and no virus type was identified for 8. Of the 113 total samples, 16 (14.1%) were from BGM cells, 28 (24.7%) were from Ma-104 cells, and 2 (1.7%) were from Vero cells. Vero cells were not at all sensitive for recovery of virus from environmental samples. There was no difference between trypsin-treated or untreated BGM or Vero cells for laboratory strain poliovirus type 1; however, trypsin-treated Ma-104 cells yielded isolates more frequently than BGM cells. The statistical analysis shows a significant difference ($P < 0.0001$).

Table 1 shows the virus types isolated from wastewater samples in each of the host cell culture systems. No poliovirus type 2 was isolated, and poliovirus type 1 was more frequently isolated than poliovirus type 3. Reovirus type 2 was identified only with Ma-104 cells. The unidentified viruses were as frequent with BGM (7%) as with Ma-104 (3.4%) ($P > 5\%$). Furthermore, there was no difference for isolating group B coxsackieviruses with BGM (4.9%) or Ma-104 (2.6%) ($P > 5\%$).

TABLE 2. Virus isolated simultaneously from positive samples with the three cell systems

Cell system	Virus isolated from sample[a]:						
	21	24	26	65	75	81	83
Ma-104	+ P 1	+ UI	+ CB 5	+ CB 2	+ UI	+ P 1	+ CB 5
BGM	−	+ UI	+ CB 5	+ CB 2	+ UI	+ P 1	+ CB 5
Vero	+ P 1	−	−	−	−	−	−

[a] UI, Unidentified; CB, coxsackievirus B; P, poliovirus.

Virus isolated simultaneously from samples. Table 2 shows the samples for which a virus was revealed with at least two cell systems. Only one sample yielded a virus in Vero and Ma-104 cells, and six yielded a virus in Ma-104 and BGM cells, two of which were unidentified. No virus from one sample was revealed in all three systems.

DISCUSSION

The BGM line of continuous African green monkey kidney cells established by Barron et al. (2) and now widely used has been highly recommended by some workers, i.e., Dahling et al. (6), because of its high sensitivity for the recovery of viruses from water. Thus, it was tested for the isolation of human enteric viruses from sewage, sediment, and shellfish samples (8, 11). However, other workers have shown BGM cells to be unsatisfactory for isolating viruses. For Schmidt et al. (18, 19), BGM cells were more sensitive only for poliovirus and group B coxsackievirus. Ridinger et al. (16) showed that these cells are inferior to other monkey kidney cell lines for isolating reoviruses from sewage. The large number of samples with an unconfirmed cytopathic effect found with BGM (7%) underlines the problem of false plaques observed by Schmidt et al. (19). The use of Ma-104 in the present study at passage levels 60 to 92 allowed us to observe a different spectrum of enteric viruses than did BGM cells. Sobsey et al. (23) reported similar results. However, our study has not confirmed that the number of virus isolates from samples is lower on Ma-104 cells than on BGM cells. Furthermore, with Ma-104 cells we isolated mixtures of viruses; this simultaneous isolation of different viruses is less frequent with BGM. The percentage of reovirus specimens obtained is equal to that found by Irving and Smith (9) after a 7- to 9-day incubation with an effluent from an activated sludge purification plant. On the other hand, our isolation rate is much lower than that found with BSC 1 by Sattar and Westwood (17).

The Vero cell line was included in this study because it supports the replication of certain enteroviruses contained in fecal samples. It is clearly unsatisfactory for the recovery of viruses from environmental samples, as has been suggested elsewhere by Charrier et al. (5).

It thus appears that, because of their larger sensitivity spectrum, easy maintenance, and resistance to toxic effects, trypsin-treated Ma-104 cells may be recommended in conjunction with other cell lines for the detection of viruses from environmental samples.

ACKNOWLEDGMENTS

We thank A. Chauvin for excellent technical assistance and C. Quille for efficient typing.

LITERATURE CITED

1. **Agbalika, F., P. Hartemann, M. Brigaud, and J. M. Foliguet.** 1983. Enterovirus circulation in wastewater of the Lorraine area: evaluation of the survey from 1976 to 1981. Rev. Epidem. Santé Publ. **31**:209–221.
2. **Barron, A. L., C. Olsheosky, and M. M. Cohen.** 1970. Characteristics of the BGM line of cells from African green monkey kidney. Arch. Virusforsch. **32**:389–392.
3. **Buras, N.** 1974. Recovery of viruses from wastewater and effluent by the direct inoculation method. Water Res. **8**:19–22.
4. **Buras, N.** 1976. Concentration of enteric viruses in wastewater and effluent: a two year survey. Water Res. **10**:295–298.
5. **Charrier, F., D. Beytout, H. Laveran, and R. Cluzel.** 1977. Surveillance des virus dans les eaux usées urbaines. Considérations techniques et épidémiologiques. Med. Mal. Infect. **7**:55–60.

6. **Dahling, D. R., G. Berg, and D. Berman.** 1974. BGM, a continuous cell line more sensitive than primary Rhesus and African green kidney cells for the recovery of viruses from water. Health Lab. Sci. **11**:275–282.
7. **Foliguet, J. M., L. Schwartzbrod, and O. G. Gaudin.** 1966. La pollution virale des eaux usées de surface et d'alimentation. Bull. Org. Mond. Santé **35**:737–749.
8. **Gerba, C. P., and S. M. Goyal.** 1978. Detection and occurrence of enteric viruses in shellfish: a review. J. Food Prot. **41**:743–754.
9. **Irving, L. G., and F. A. Smith.** 1981. One-year survey of enteroviruses, adenoviruses, and reoviruses isolated from effluent at an activated-sludge purification plant. Appl. Environ. Microbiol. **41**:51–59.
10. **Joret, J. C., J. C. Block, P. Hartemann, and J. M. Foliguet.** 1981. Virus recovery from wastewater using microfiberglass filters, p. 30–34. *In* M. Goddard and M. Butler (ed.), Viruses and wastewater treatment: proceedings of an international symposium held at the University of Surrey, Guildford, 15–17 September 1980. Pergamon Press, Oxford.
11. **LaBelle, R. L., C. P. Gerba, S. M. Goyal, J. L. Melnick, I. Cech, and G. F. Bogdan.** 1980. Relationships between environmental factors, bacterial indications, and the occurrence of enteric viruses in estuarine sediments. Appl. Environ. Microbiol. **39**:588–596.
12. **Leloup, D., G. A. Denoyel, A. Gaspar, and J. Vial.** 1979. Etude de la contamination virale des eaux de la région Rhône Alpes entre 1975 et 1977. Rev. Inst. Pasteur Lyon **12**:371–386.
13. **Martins, M. T., L. A. Soares, E. Marques, and A. G. Molina.** 1982. Human enteric viruses isolated from effluent of sewage treatment plants in S. Paulo, Brazil. *In* Proceedings of the 11th IAWPR, Pretoria, Republic of South Africa.
14. **Marzouk, Y., S. M. Goyal, and C. P. Gerba.** 1971. Relationship of viruses and indicator bacteria in water and wastewater of Israel. Water Res. **4**:1585–1590.
15. **Payment, P., Y. Larose, and M. Trudel.** 1979. Poliovirus as indicator of virological quality of water. Can. J. Microbiol. **25**:1212–1214.
16. **Ridinger, D. N., R. S. Spendlove, B. B. Barnett, D. B. George, and J. C. Roth.** 1982. Evaluation of cell lines and immunofluorescence and plaque assay procedures for quantifying reoviruses in sewage. Appl. Environ. Microbiol. **43**:740–746.
17. **Sattar, S. A., and J. C. N. Westwood.** 1977. Isolation of apparently wild strains of poliovirus type 1 from sewage in the Ottawa area. Can. Med. Assoc. J. **116**:25–27.
18. **Schmidt, N. J., H. H. Ho, and E. H. Lennette.** 1976. Comparative sensitivity of the BGM cell line for isolation of enteric viruses. Health Lab. Sci. **13**:115–117.
19. **Schmidt, N. J., H. H. Ho, J. L. Riggs, and E. H. Lennette.** 1978. Comparative sensitivity of various cell cultures systems for isolation of viruses from wastewater and fecal samples. Appl. Environ. Microbiol. **36**:480–486.
20. **Sellwood, J., and J. V. Dadswell.** 1980. Viruses isolated from sewage 1974–1977, p. 15–17. *In* M. Goddard and M. Butler (ed.), Viruses and wastewater treatment: proceedings of an international symposium held at the University of Surrey, Guildford, 15–17 September 1980. Pergamon Press, Oxford.
21. **Sigel, M. M., D. F. Rippe, A. R. Beasley, and M. Dorsey, Jr.** 1976. Systems for detecting viruses and viral activity, p. 139–164. *In* G. Berg, H. L. Bodily, E. H. Lennette, J. L. Melnick, and T. G. Metcalf (ed.), Viruses in water. American Public Health Association, Inc., Washington, D.C.
22. **Sobsey, M. D.** 1976. Methods for detecting enteric viruses in water and wastewater, p. 89–127. *In* G. Berg, H. L. Bodily, E. H. Lennette, J. L. Melnick, and T. G. Metcalf (ed.), Viruses in water. American Public Health Association, Inc., Washington, D.C.
23. **Sobsey, M. D., S. E. Oglesbee, D. A. Wait, and A. F. Meinhold.** 1981. Cell culture procedures: the use of Ma 104, a continuous cell line for the isolation of human enteric viruses from environmental samples, p. 32. *In* N. A. Clarke (ed.), Public health aspects of viruses in water, vol. 8. U.S. Environmental Protection Agency, Cincinnati, Ohio.