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Effect of Biocides on MS2 and K Coliphages

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Several biocides commonly used in disinfection processes as antibacterial and antifungal agents were tested for activity against MS2 and K coliphages. MS2 was resistant to most biocides; only glutaraldehyde (0.5%) and peracetic acid (1%) achieved a 4-$\log_{10}$ titer reduction in 20 min. In contrast, K phage was sensitive to most biocides, being resistant only to phenol (2%) and chlorhexidine (1%).

Coliphage MS2 has been investigated as a potential indicator for contamination of water by sewage (7), for its sensitivity to disinfectants such as chlorine and ozone (2, 8, 13, 14), and to help establish new viral models for human pathogens (4). When treated with some disinfectants, MS2 behaves similarly to poliovirus (6), reinforcing the idea of replacing the human enterovirus assays with MS2 for studying resistance mechanisms or the virucidal activity of biocides. In contrast, K bacteriophage has been less widely investigated. It contains single-stranded DNA, possesses a tailed structure, and is larger than MS2. In the present study, MS2 and K coliphages were tested at different pHs against a wider range of biocides than used by previous workers as part of a broadly based study to better understand the mechanisms of the virucidal action of chemicals. The active agents are commonly used in disinfection processes because of their antibacterial and antifungal properties. However, less is known about the virucidal efficacy of such biocides (3).

MS2 was grown to a high titer on *Escherichia coli* 9481. K phages were grown on *E. coli* D837. A 1-ml volume of an overnight culture of the host cells (approximately 10^9 CFU/ml) was added to 100 ml of nutrient broth (Oxoid) and incubated at 37°C in a water bath under constant agitation. After 3 h of incubation, 1 ml of a phage suspension (5×10^9 to 2×10^{10} PFU/ml) was added to the appropriate host and incubated overnight at 37°C. The mixture was then centrifuged (Centaur 2 centrifuge; Fisons) for 30 min at 4,000 rpm, and the supernatant was filtered through a 0.2-μm-pore-size membrane filter (Whatman) before being stored at 4°C. All of the agar used for the MS2 experiments contained 0.1% peptone (Difco) and 5×10^{-4} M calcium chloride ($CaCl_2$; BDH, Poole, Dorset, United Kingdom).

For phage titration, the molten, soft overlayer agar technique was used. A 150-μl sample (10^9 CFU/ml) of an overnight culture of the host bacterial strain was mixed with 100 μl of the phage in 3 ml of 0.65% molten nutrient agar (Oxoid) at 45°C. The contents were gently poured over the surface of an agar plate. After incubation for 18 to 20 h at 37°C, the plaques were counted and the results were expressed as PFU per milliliter.

The virucidal suspension test method described in reference 1 was adapted for the use of bacteriophages (10). The disinfectants tested (with the corresponding neutralizers) were 2% phenol, pHs 4 to 8 (phosphate-buffered saline [PBS]); 0.1 and 1% peracetic acid, pHs 1.5 to 4.5 (0.1% sodium thiosulfate); 0.05% cetylpyridinium chloride, pHs 5 to 9 (0.75% azolectin in 5% Tween 80); 1% chlorhexidine diacetate, pHs 5 to 9.5 (0.75% azolectin in 5% Tween 80); 0.5 and 1% glutaraldehyde, pHs 5 to 10 (2% glycine); 100 and 70% ethanol (PBS); and 100 to 10% isopropanol (PBS). A 1-ml volume of a bacteriophage suspension (10^9 to 10^{10} PFU/ml) was mixed with 9 ml of a biocide at the appropriate pH at 25°C. A 0.1-ml sample was removed at intervals (1 to 20 min) and mixed with 9.9 ml of the appropriate neutralizing solution. The mixture was serially diluted in PBS. A 100-μl volume of the appropriate dilution mixed with 150 μl of an 18- to 20-h overnight host cell broth culture was added to 3 ml of molten soft agar at 45°C. This was then poured onto an agar plate and incubated for 24 h at 37°C. Plaques were counted, and results were expressed as PFU per milliliter. Preliminary experiments demonstrated that bacterial hosts and phages were insensitive to the neutralizers used and that the neutralizing systems were effective in quenching biocide activity. All experiments were carried out in quintuplicate. Two-way and one-way analyses of variance were performed by using Minitab statistical software. There was no significant difference in the distribution of the variances of the different sets of data compared to the F distribution at the 95% confidence level. Examples of results are provided in Tables 1 and 2. A disinfectant was considered to be effective against the coliphages when it produced a 4-$\log_{10}$ reduction of the phage titer (1). Only glutaraldehyde (pHs 8 to 10) at a concentration of 0.5 or 1% achieved a 4-$\log_{10}$ reduction of the MS2 titer within 20 min. Peracetic acid (1%) and ethanol (70%) reduced the phage titer by 3.95 and 3.68 $\log_{10}$, respectively. Cetylpyridinium chloride (0.05%) produced a 2.7-$\log_{10}$ reduction at pH 7, whereas chlorhexidine (1%), isopropanol (100%), and phenol (2%) were not effective against MS2, irrespective of the pH.

Neither chlorhexidine (1%) nor phenol (2%) at any pH inactivated K phage within 20 min. However, the other biocides investigated produced a 4-$\log_{10}$ reduction within this period; e.g., ethanol (70%), isopropanol (100%), and peracetic acid (1%) did so within 1 min, glutaraldehyde (0.5%) did so within 2.5 min, and cetylpyridinium chloride (0.05%, pH 7) did so within 5 min.

Because its resistance to biocides is greater than that of *E. coli* and because of its presence in sewage, MS2 coliphage has been used as an indicator organism for contamination of water by sewage in some European and other countries (7). Its adenovirus, parvovirus-like structure and its RNA genome suggest that MS2 may be used as a model for hepatitis A virus and poliovirus in the investigation of viral inactivation and mechanisms of resistance (5).

In contrast, the K coliphages were more sensitive to the

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TABLE 1. Virucidal activities of test biocides against MS2

Biocide concn (%)	pH	Contact time[a] (min)	Mean $\log_{10}$ reduction (PFU/ml) ± SD
Glutaraldehyde			
1	8	7.5	4.132 ± 0.016
0.5	8	10	5.189 ± 0.382
Cetylpyridinium chloride, 0.05	7	20	2.729 ± 0.021
Chlorhexidine diacetate, 1	9	20	1.134 ± 0.057
Peracetic acid, 1	1.5	20	3.949 ± 0.775
Phenol, 2	4	20	0.509 ± 0.239
Ethanol			
100		20	1.533 ± 0.135
70		20	3.675 ± 0.123
Isopropanol, 100		20	1.503 ± 0.182

[a] Minimum contact time required for maximum inactivation.

biocides investigated. However, the nature of these coliphages (an equal mixture of K1, K2, K3, K4, and K5) gave K phage a wide range of bacterial infectivity (at least five bacterial serotypes) and offered a reliable and easily readable system because it forms large, clear plaques. Thus, K phage could be used for preliminary screening of the antiviral activity of active ingredients.

TABLE 2. Virucidal activities of test biocides against K bacteriophage

Biocide concn (%)	pH	Contact time[a] (min)	Mean $\log_{10}$ reduction (PFU/ml) ± SD
Glutaraldehyde, 0.5	10	2.5	4.622 ± 0.333
Cetylpyridinium chloride, 0.05	7	5	4.636 ± 0.362
Chlorhexidine diacetate, 1	7	20	0.544 ± 0.072
Peracetic acid			
0.1	2.5	2.5	4.517 ± 0.145
1	2.5	1	4.903
Phenol, 2	4	20	0.436 ± 0.059
Ethanol			
100		1	5.129 ± 0.207
70		1	4.885 ± 0.188
Isopropanol			
100		1	4.780 ± 0.277
20		20	0.185 ± 0.109
10		20	0.098

[a] Minimum contact time required for maximum inactivation.

Repetitive results were obtained with the two test coliphages. Further, they are easy to use, they are safe because they are nonpathogenic, and experimental costs are minimal.

The wide range of bacteriophages that can be used offers a powerful tool for a large number of different studies: for contamination investigations, for screening of new antiviral active agents (9, 11, 12), and for exploration of viral infection and resistance mechanisms. In addition, bacteriophages can easily be genetically modified. The sizes, structures, and genomes of the two coliphages we studied are very different, but we cannot as yet explain why cetylpyridinium chloride (0.05%) was highly active against the coliphages whereas chlorhexidine (1%), another cationic agent, was not.

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