VIRUCIDAL ACTIVITY OF A NEW PHOSPHORIC ACID-WETTING AGENT SANITIZER AGAINST BACTERIOPHAGE OF STREPTOCOCCUS CREMORIS

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Dairy plant sanitation procedures represent one of the chief factors affecting incidence of starter failure caused by bacteriophage in cultured milk products. Consequently there is much interest in the virucidal activity of the sanitizing agent used. A new phosphoric acid-wetting agent sanitizer (PAWA) which consists chiefly of orthophosphoric acid plus nonionic and anionic surface active agents, has been developed for use as a dairy sanitizer. Studies were conducted to determine the ability of this new sanitizer to destroy bacteriophage active against a representative lactic streptococcus. Its virucidal activity also was compared with other commonly used sanitizers such as hypochlorites, iodophors, and quaternary ammonium compounds (QACs).

Streptococcus cremoris phage strain 144F was employed in the virucidal trials as a representative phage. The virucides selected were of the type commonly employed in general dairy and food plant sanitizing procedures. In addition to the new PAWA sanitizer the following were used: calcium hypochlorite Ca(OCl)₂ and sodium hypochlorite NaClO, several iodophors (I-1, I-2, and I-3), a product consisting of potassium iodide and chloramine-T, also known as diatomic iodine (DI), and a QAC (alkyl dimethyl benzyl ammonium chloride).

The PAWA sanitizer was diluted according to the manufacturer’s instructions and approximate concentration established with 0.35 N sodium hydroxide solution. The standard thiosulfate titration (1) for determining available chlorine was used for the hypochlorites and the iodophors were titrated to a colorless end point with standard thiosulfate. QAC concentrations were determined by the method of Furlong and Elliker (3). Virucide solutions were diluted with distilled or USDA (2) buffered water of 590 ppm hardness.

The technique for evaluating virucidal activity was that used by Watkins, Hays and Elliker (5) with the following modifications: A M/50 phosphate buffer solution at pH 7.2 was used to inactivate the PAWA sanitizer. Thiosulfate inactivator solutions for the hypochlorites and iodophors and lecthin-Tween 80 inactivator solutions for the QAC were prepared in M/100 phosphate buffer at pH 7.2.

Whey filtrates of a phage strain of S. cremoris 144F were prepared as described by Parker and Elliker (4) with the following variations: The whey filtrate containing the propagated phage was passed through a Seitz filter instead of being filtered through a Selas candle. The cell free filtrate was transferred to a sterile bottle containing an excess of CaCO₃ to neutralize whey acidity. A 1:100 dilution of this filtrate in sterile water was used for virucidal trials. Distilled water was used for filtrate dilution in those trials where the virucide was tested in distilled water and USDA water was used to dilute the phage in trials where the virucides were tested in USDA water. The plaquing procedure was the same as described by Watkins, et al. (5).

RESULTS

Results shown in Tables are representative of those obtained with three different strains of bacteriophage specific for lactic streptococci. Those represented were obtained using phage for S. cremoris 144F.

Table 1 shows a comparison of various germicides in buffered hard water against S. cremoris phage. The recommended dilution of 1-128 to give 200 ppm of the active ingredients of the PAWA sanitizer completely inactivated the phage within 15 seconds. The QAC in a concentration of 50 ppm and 25 ppm of both Ca(OCl)₂ and NaOCl also inactivated the phage in 15 seconds. The I-1 in a concentration of 25 ppm showed a slower rate of virucidal activity and failed to completely inactivate the phage after 60 seconds of exposure. The DI compound in 25 ppm failed to inactivate the phage in 15 seconds but was effective after a 60 second exposure period.

Studies were conducted to compare differences in activity of several virucides when distilled and buffer-
iodophor compounds gave complete inactivation of the phage after a 60-second exposure period.

**Table 3 — Virucidal Activity of Various Concentrations of a Phosphoric Acid-Wetting Agent Sanitizer Against Streptococcus cremoris Phage Strain 144F in Distilled and USDA Test Water**

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Type of water</th>
<th>Final pH</th>
<th>Plaque count after following exposure times:</th>
<th>15 sec.</th>
<th>30 sec.</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5</td>
<td>Dist.</td>
<td>2.65</td>
<td>1.8x10&lt;sup&gt;0&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>Dist.</td>
<td>2.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>Dist.</td>
<td>2.2</td>
<td>1.2x10&lt;sup&gt;0&lt;/sup&gt;</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>100</td>
<td>Dist.</td>
<td>1.95</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>200</td>
<td>Dist.</td>
<td>1.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>USDA</td>
<td>2.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Initial count of phage-germicide mixture: 14 x 10<sup>6</sup> per ml. Plaque count after exposure represents number per ml of a 1:10 dilution of phage-germicide mixture.

Trials were made with the PAWA sanitizer (Table 3) to determine how much it could be diluted and still retain virucidal properties. When diluted to a concentration of 1.2048 (approximately 12.5 ppm) in distilled water, it inactivated the phage within 15 seconds. When buffered hard water was used as the diluent, a dilution of 1-512 (50 ppm) inactivated the phage in 15 seconds, but 1.1024 (25 ppm) failed to inactivate the phage after a 60-second exposure. Activity in the two types of water appears directly related to the pH of the final germicide-phage solution. In distilled water the pH of the final solution was less than 3.0 in the highest dilution used. In the buffered water there was a marked increase in pH between the 1-512 dilution and the 1-1024 dilution. The former was pH 3.0 while the pH of the latter was 5.8.

**Discussion**

Results of this study and an earlier investigation (5) suggest that pH of solution represents a critical factor in determining rate of destruction of bacteriophage by some acid-sanitizing agents. Hypochlorites in this and previous studies demonstrated a high rate of activity against all lactic streptococcus bacteriophage strains employed. Whether or not QAC activity may be enhanced by use of an acid product was not established in this investigation.

Initial concentration of bacteriophage particles might be expected to affect efficiency of any specific concentration of a sanitizer within certain limits. Bearing this factor in mind, the critical pH for rapid destruction of bacteriophage for the acid-wetting
agent in these studies appeared to be in the range of 2.0 to 3.0. It is possible also that surface agents employed in the acid-wetting agent sanitizer contributed to phage inactivation. In general, results suggested that a concentration of 200 ppm, as recommended for use dilution of this product, maintained the pH in the range of 2.0 to 2.35 which should assure a high rate of phage destruction. Dilution of the product to sublethal levels and trials in unbuffered and synthetic hard water merely served to emphasize the significance of pH in determining activity of the phosphoric acid-wetting agent.

**Summary**

A comparison was made under laboratory conditions of the relative effectiveness of a new PAWA sanitizer and representative hypochlorites, QAC, and iodophor compounds in the destruction of lactic streptococcus bacteriophage. The effect of dilution and buffered hard water on the activity of the new sanitizing agent also was studied.

Both NaOCl and Ca(OC1)2 in a concentration of 25 ppm completely inactivated the phage of S. cremoris 144F during a 15-second exposure period. The iodophor compounds showed a slower rate of activity when used in a concentration of 25 ppm. In distilled water a 60-second exposure period was required for complete destruction of the phage and in buffered hard water the efficiency was greatly decreased. A concentration of 50 ppm QAC was effective in a 15-second exposure period.

The PAWA sanitizer was effective in concentrations as low as 12.5 ppm during a 15-second exposure period in distilled water but in buffered hard water a concentration of 50 ppm was required to inactivate the phage in 15 seconds. The results suggest that this sanitizer when used in recommended concentration of 200 ppm should provide an effective agent for destruction of bacteriophage on dairy equipment.

**References**