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Effectiveness of Commercial Germicide Products Against the Ropy Slime-Producing Lactic Acid Bacteria

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ABSTRACT

The effectiveness of certain commercial germicides against ropy slime-producing lactic acid bacteria was studied using the 5-5-5-suspension test and a tray test which simulated surface disinfection conditions in actual use. The rate of the destruction of the bacteria was lower in the tray test, and the bacteria seemed to be more resistant to the germicides on a steel surface than in vitro. The sanitizer products proved to be more effective than the detergent-sanitizer products. Quaternary ammonium products and an acid sanitizer with hydrogen peroxide were more effective than products containing chlorine compounds and polyhexamethylene biguanide chloride. The effectiveness of hypochlorite products appeared to be dependent on the concentration of available chlorine. The resistance of the ropy slime-producing bacteria may prove problematic if ineffective germicides are used. The use of detergent sanitizers and low-concentration hypochlorite products is not recommended.

During the last few years, the formation of ropy slime on vacuum-packed cooked sausages and cooked whole meat products has been a problem in the Finnish meat industry. The slime is often formed before the sell-by date, and consumers find the appearance of a slimy product very offensive (*7,8*). This ropiness has been shown by Korkeala et al. (*8*) to be caused by lactic acid bacteria capable of producing ropy slime. Three different slime-producing lactic acid bacteria groups, two of which were lactobacilli, and one of which belonged to the genus *Leuconostoc*, have been isolated (*8*).

Several studies (*1,4,10,13*) have found that cooked meat products become recontaminated with lactic acid bacteria during handling after cooking; these bacteria have also been found in the rooms and equipment of meat processing plants (*4,6,13*). These rooms can thus act as a source of the bacterial contamination. The aim of the sanitation at a food processing plant is to reduce the bacterial load of the rooms and minimize contamination of the products with microorganisms (*9*). According to a survey of the Finnish meat industry, sanitation was considered to be one of the most important ways of preventing the ropiness problem (*14*).

The purpose of the present study was to evaluate the effectiveness of commercial germicide products in destroying lactic acid bacteria which produce ropy slime. The products tested were those commonly used in the Finnish food industry. The specific activity of the products were tested in vitro using the 5-5-5-suspension test, and their effectiveness under simulated plant conditions was evaluated by a tray test.

MATERIALS AND METHODS

Bacterial strains

Three different lactic acid bacteria strains producing ropy slime were used. The strains were those isolated from ropy meat products by Korkeala et al. (*8*); all of the strains were capable of causing ropiness on vacuum-packed, cooked meat products. Strain A210 was from group 1, strain Cl from group 2, and strain Dl from group 3 of Korkeala et al. (*8*). Strains A210 and Cl were homofermentative lactobacilli, and strain Dl was a *Leuconostoc* strain.

Germicide products

Six commercial detergent sanitizers and eight sanitizers were tested. The products studied, their antimicrobial compounds, and the concentrations of the compound in the solutions tested are listed in Table 1. The concentration tested was the lowest recommended by the manufacturer. The solutions of the disinfectants were prepared in physiological NaCl solution in the 5-5-5-suspension test and in distilled water in the tray test. The solutions were prepared just before the test runs.

5-5-5-suspension test

The 5-5-5-suspension test was performed according to the procedure given by the Netherlands phytopharmaceutical committee (*5*), with the following modifications: The bacteria used were the three bacterial strains listed above. The bacteria were grown on MRS-agar (Oxoid, Basingstoke, England) plates anaerobically in an anaerobic jar using H_2 + $C0_2$ generating kit (Oxoid) at 20°C for 3 d. The strains were subcultured in MRS-broth (Difco, Detroit, MI) and incubated overnight at 20°C. The culture was then centrifuged; the sediment was washed once with diluent (Bovine albumin; Sigma, St. Louis, MO (0.03%, NaCl 0.9%) and suspended in the same diluent to obtain a concentration of approximately 10^9 cells/ml. A 100-ml Erlenmeyer flask was filled with 24 ml of the germicide solution. A control flask was filled with 24 ml of physiological NaCl solution. The flasks were placed in a 20°C water bath. Two ml of bovine albumin solution (Bovine albumin 1.5 %) was added to 2 ml of the bacterial suspension and incubated 2 min at 720°C; 1 ml of this new solution was then added to the flask containing the germicide solution and to the control flask. After that, the flasks were incubated in a water bath

TABLE 1. *The antimicrobial compounds in the germicide products tested, and the concentration of the compound in the solution tested.*

Germicide product	Antimicrobial compound	Concentration of the compound in solution
Detergent sanitizers (DS)		
DS 1	Na-dichloroisocyanurate	0.06%
DS 2	Na-hypochlorite	Available chlorine 0.017%
DS 3	Na-hypochlorite	Available chlorine 0.07%
DS 4	Na-hypochlorite	Available chlorine 0.017%
DS 5	Na-hypochlorite	Available chlorine 0.02%
DS 6	Cocobenzyldimethyl ammonium chloride	0.027%
	Dimethylcoco ammonium betaine	0.027%
Sanitizers (S)		
S1	Alkyldimethylbenzyl ammonium chloride	0.022%
	Alkylmethylethylbenzyl ammonium chloride	0.022%
S2	Alkyldimethyl ammonium chloride	0.014%
	Alkylmethylethylbenzyl ammonium chloride	0.014%
	Polyhexamethylene biguanide chloride	0.023%
S 3	Na-hypochlorite	Available chlorine 0.09%
S 4	Alkyldimethylbenzyl ammonium chloride	0.05%
S 5	Benzyldimethylalkyl ammonium chloride	0.1%
S 6	Na-hypochlorite	Available chlorine 0.024%
S 7	Peracetic acid	0.018%

at 20°C for 5 min. Then 1 ml liquid of each flask was transferred to 9 ml of inactivation solution containing soya lecithin (NBCo Biochemicals, Cleveland, OH) 0.3%; polysorbate 80 (Oriola, Espoo, Finland) 3.0%; sodium thiosulfate (Merck, Darmstadt, RG) 0.5%; L-histidine (Merck, Darmstadt, FRG) 0.1%; and 0.25 N phosphate buffer 10%. The inactivation solutions were held for 5 min at room temperature. Decimal dilutions were then made in physiological NaCl solutions and plated into MRS-agar (Oxoid). The plates were incubated anaerobically for 5 d at 20°C. The inactivation of the germicides was tested by adding 1 ml of the bacterial solution from the control flask to 9 ml of the inactivation solution to which 1 ml of the germicide solution had just been added. The dilution and plating were done as above. Inactivation was tested using strain A210. The test was run twice for each germicide with each of the three ropy slime-producing bacteria.

Tray test

The bacterial strains were grown and subcultured as above. Ten ml of the bacterial culture was inoculated on a stainless steel tray (size 30 x 25 cm^2). The culture was spread evenly over the whole tray with a sterile glass rod. The tray was allowed to dry at room temperature for 1 h. The bacterial count on the surface was 10^4-10^6 CFU/50 cm^2. The surface of the tray was sprayed with 20 ml of the germicide solution, which was allowed to work for 10 min at room temperature. The extra liquid was then poured away and the tray was flooded for 5 min with the same inactivation solution as above. A control tray was handled in the same way with the exception of the germicide spraying. The surviving organisms were recovered by rubbing an area of 50 cm^2 of the surface vigorously with a cotton ball held in tweezers. The cotton ball was placed in a bottle containing 10 ml of physiological NaCl solution, and the bottle shaken for 2 min. The samples were then diluted l0-fold in physiological NaCl solution and plated as above. Each germicide was tested twice with each slime-producing bacterial strain.

Calculating the results

The microbicidal effect (ME) of each germicide product was calculated using the following equation:

$$ME = \log A - \log B$$

in which A = the bacterial count in the test system without germicide and

B = the bacterial count in the test system with germicide

In the 5-5-5-suspension test the product was considered effective if the ME value was over 5 (*5*).

The percentage reduction of the microbial count was calculated as an average of the percentage reduction in the two test runs.

RESULTS

The ME values and the percentage reduction values of the detergent sanitizers and the sanitizers in the 5-5-5-suspension test and in the tray test are presented in Tables 2 and 3, respectively. In the 5-5-5-suspension test, three detergent sanitizers and two sanitizers showed ME values lower than 5 in some of the six test runs. None of these products were ineffective in all the test runs. The products were usually effective against the bacterial strain being tested in one test run but not in the other. In the tray test, the ME values and the percentage reduction values were usually lower than in the 5-5-5-suspension test. The lowest percentage reduction values in the tray test were observed with the germicides DS 2, DS 4, DS 5, S 6, and S 8, which also had ME values lower than 3. The low values in the

TABLE 2. *The microbicidal effect (ME) and the percentage reduction values of the detergent sanitizers used in the 5-5-5-suspension test, and in the tray test, against the ropy slime-producing lactic acid bacteria.*

Detergent sanitizer	5-5-5-suspension test Bacterial strain A210	C1	D1	Tray test Bacterial strain A210	C1	D1
DS 1	4.4[a]	3.7	>6.3	>4.6	>4.5	>6.4
	>5.7[a]	>5.7	6.1	>5.6	>4.5	>5.6
	99.998[b]	99.990	OV	OV	OV	OV
DS 2	>6.3	2.8	4.6	3.4	1.3	>5.5
	5.5	>6.2	2.3	4.1	2.6	2.5
	OV	99.920	99.742	99.98	97.32	99.86
DS 3	5.3	>6.3	5.4	5.0	3.8	>6.3
	6.1	>6.0	>6.5	>5.2	4.4	4.8
	OV	OV	OV	OV	99.99	OV
DS 4	>6.2	>5.9	>6.6	3.1	1.7	2.4
	3.4	>6.5	5.9	1.2	1.7	4.8
	99.978	OV	OV	96.95	97.93	99.81
DS 5	>6.4	>6.1	>6.4	>5.1	1.1	3.0
	>6.3	>6.3	>6.6	0.9	1.6	4.2
	OV	OV	OV	92.77	94.17	99.99
DS 6	>6.4	>6.2	>6.0	>5.9	>4.5	4.8
	>6.5	>6.3	>6.9	>5.3	>5.6	5.3
	OV	OV	OV	OV	OV	99.99

[a]ME values of the two test runs; ME <5 in the 5-5-5-suspension test is considered as ineffective.
[b]Average percentage reduction value for the two runs; values over 99.999% in the 5-5-5-suspension test and values over 99.99% in the tray test are marked with OV.

TABLE 3. *The microbicidal effect (ME) and the percentage reduction values of the sanitizers used in the 5-5-5-suspension test, and in the tray test, against the ropy slime-producing lactic acid bacteria.*

Germicide product	5-5-5-suspension test Bacterial strain A210	C1	D1	Tray test Bacterial strain A210	C1	D1
S 1	>6.4[a]	>6.2	>5.6	5.0	>5.2	>6.8
	>6.4[a]	>6.0	6.5	>5.6	>5.8	5.4
	OV[b]	OV	OV	OV	OV	OV
S 2	>5.3	>6.3	>6.4	>4.2	4.2	>5.5
	>6.7	>6.3	>6.4	>5.3	>5.2	>6.8
	OV	OV	OV	OV	OV	OV
S 3	>5.6	5.6	>6.0	>5.0	>5.0	>6.2
	>6.6	>6.3	>6.4	4.6	>5.2	5.2
	OV	OV	OV	OV	OV	OV
S 4	>6.2	>5.9	>6.6	4.8	5.8	>5.6
	>6.1	>6.7	>6.3	>4.2	>4.0	>5.5
	OV	OV	OV	OV	OV	OV
S 5	>5.7	>6.1	>6.7	4.8	>4.0	>5.5
	>6.5	>6.8	>6.7	>4.2	>5.0	>5.1
	OV	OV	OV	99.99	OV	OV
S 6	>5.8	>6.2	3.5	4.2	1.3	2.0
	2.4	>6.2	6.3	4.0	5.3	1.9
	99.805	OV	99.984	99.99	97.43	98.90
S 7	>5.4	>5.7	>5.7	>5.9	>4.5	>5.4
	>6.2	>6.2	>6.6	>5.3	5.4	>6.0
	OV	OV	OV	OV	OV	OV
S 8	>6.2	>6.3	5.2	>5.3	2.4	5.8
	4.4	6.6	5.4	2.5	>5.2	4.5
	99.998	OV	OV	99.85	99.77	99.99

[a]ME values of the two test runs; ME <5 in the 5-5-5-suspension test is considered as ineffective.
[b]Average percentage reduction value for the two runs; values over 99.999% in the 5-5-5-suspension test and values over 99.99% in the tray test are marked with OV.

tray test were found in the same germicides, which proved ineffective in the 5-5-5-suspension test, with the exception of the germicide DS 1 which was ineffective in the 5-5-5-suspension test but not in the tray test, and the germicide DS 5 which was ineffective only in the tray test. In both tests low ME values were observed more often with the detergent sanitizers than with the sanitizers.

The most effective detergent sanitizer products were DS 6, which contained quaternary ammonium compounds and an amphoteric compound, and DS 3, which had the highest concentration of Na-hypochlorite. The other three detergent sanitizer products which contained Na-hypochlorite, and the germicide DS 1, which contained Na-dichloroiso-cyanurate, were all ineffective to some extent.

The two ineffective sanitizer products were S 6, which contained Na-hypochlorite, and S 8 with polyhexamethylene biguanide chloride. The other sanitizers were effective in both the tests.

No great differences between the ropy slime-producing bacterial strains were found in regard to sensitivity to the germicides being tested. Those germicides with low ME values were found to be ineffective against at least two of the three bacterial strains studied. The inactivation solution used was found to neutralize the germicides studied.

DISCUSSION

In this study primarily the same products were ineffective in the 5-5-5-suspension test and in the tray test. However, the rate of the destruction of the bacteria was lower in the tray test and the ropy slime-producing bacteria seemed to be more resistant to germicides on the steel surface than under in vitro conditions. Similar results have also been reported by other workers. Mosley et al. (*11*) demonstrated that the food spoilage, indicator and pathogenic organisms which they studied showed greater resistance to germicides when inoculated onto stainless steel than when tested in vitro. Mustapha and Liewen (*12*) have reported that *Listeria monocytogenes* exhibit more resistance to sanitizers on stainless steel surfaces than under in vitro conditions.

The efficiency of the germicides used in the tray test might also be decreased by the slime produced by the bacteria in MRS-broth. This slime was obviously present in much larger quantities in the tray test, in which the plain culture broth was used, than in the 5-5-5-suspension test where the washing of the bacterial cells probably rinsed most of the slime away. The slime may have a protecting effect against germicides; it might, e.g., mechanically protect the bacteria from contact with the sanitizing compound.

Products made for use as sanitizers were more effective against the bacteria tested than were the detergent sanitizers. The lower effectiveness of the detergent sanitizers may be due in part to the surface-active compounds used in these products. The surface-active compounds may modify the antimicrobial activity of the product. The microbicidal effectiveness can be improved, but mostly a decrease in the antimicrobial activity has been reported *(2,3,11,15)*. Mosley et al. (*11*) also found commercial iodophor detergent sanitizers less effective than iodophor products without detergents; and they, too, suggested that this difference in germicidal activity might be related to surface-active agents. In actual use at meat processing plants, it is apparently better to use detergents and sanitizers separately than to use combined detergent sanitizer products. Simultaneous cleaning and disinfection may also have other disadvantages, such as a reduction in the activity of the sanitizer in the presence of organic matter.

Products containing quaternary ammonium compounds and the product with peracetic acid and hydrogen peroxide proved to be effective against the ropy slime-producing bacteria. Some of the chlorine products and the product with polyhexamethylene biguanide chloride were ineffective. The effectiveness of the products containing Na-hypochlorite seemed to be dependent on the concentration of available chlorine in the solution being tested, since the products with the highest concentrations proved to be the most effective. It is possible that some of the ineffective Na-hypochlorite products might also have been effective if higher concentrations had been used. As all the germicides were tested using the lowest concentration recommended by the manufacturer, these recommendations are apparently not always high enough to destroy the slime-producing lactic acid bacteria. Untermann (*16*) also found that the data given by the manufacturer of the disinfectants frequently did not correspond to the concentrations actually needed to destroy the test organisms.

Since meat products become contaminated with lactic acid bacteria after the cooking process, the rooms, in which products are handled after the cooking, should be as free of the slime-producing bacteria as possible to minimize contamination. Proper cleaning and sanitizing practices are of vital importance in preventing the build-up of bacteria in these rooms. Therefore, the germicides used in sanitizing should be able to effectively destroy ropy slime-producing bacteria on surfaces in these rooms. In this study, most of the commercial germicides tested proved to be effective against these bacteria, and the survival of ropy slime-producing lactic acid bacteria should not be a problem when these products are used. Ineffectiveness was nonetheless observed in some of the products tested, and the resistance of the bacteria may cause difficulties if these products are used. It is advisable not to use such products if ropiness of the products is a problem at a plant. If still used, however, high concentrations should be employed. Since the greatest degree of ineffectiveness was observed in detergent sanitizers, their use is not to be recommended.

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