Analele Universităţii din Oradea Fascicula: Ecotoxicologie, Zootehnie şi Tehnologii de Industrie Alimentară, 2010

PHENOLIC DISINFECTANS AND THEIR EFFECTS FROM MICROBIOLOGICAL POINT OF VIEW

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Abstract

This examination was carried out to ascertain whether a change of the soap in an o-phenylphenol (45% w/w) disinfectant would have any influence on the bactericidal and fungicidal effects of the disinfectant. Tests reported that both the type and the quantity of the soap would affect the bactericidal value of Lysol B.P. For economic reasons the linseed oil soap (soap content in disinfectant 6·5%) produced by the disinfectant manufacturer was replaced with a liquid soya oil soap (soap content 3·5%) which was available commercially.

Key words: test organisms, yeast suspension, disinfectants.

INTRODUCTIONS

An alternative phenolic disinfectant, containing 9·2% (w/w) of a mixture of m-chlorocresol and benzyl-chlorophenol, was also examined with the same test organisms, to evaluate a use-dilution for the disinfectant. Two methods were employed. One was found suitable as a safe use-dilution was given three successive addition of an inoculum including a heavy load of organic matter that should simulate the practical situation. The letter test method includes geometrical dilution of the disinfectants where the germicidal concentrations are determined at a given exposure time, when using “clean” and “dirty” solutions.

MATERIALS AND METHODS

The following were used: Escherichia coli Sc, Pseudomonas aeruginosa SIFF 627, Proteus vulgaris Sr, Staphylococcus aureus SIFF 1085, phage type 80, sulphonamide and antibiotic resistant, Streptococcus faecalis Sd, and the fungi Candida albicans Sc, and Aspergillus fumigatus Sc. The strains had been isolated from pathological material, and had been used previously for disinfectant testing. The test bacteria were grown on 5% (v/v) horse blood agar in Petri dishes at 37°C. before each test, a culture of the test strain was derived from a single 24-hour-old colony. The subculture was grown for 20 h. the stock inoculum was prepared by harvesting the culture in sterile saline solution and the suspension was adjusted to a fixed optical density by means of an Eel colormeter. The "clean" inoculum was made by diluting this stock solution in saline (20 ml: 13·3 ml) to a previously determined density. This contained cs. 1-5 x 10^9 live cells/ml as determined by serial dilution technique to 10^{-6}. A known volume (0·1 ml) of the 10^{-6} dilution of the stock culture was seeded on blood agar in 4 Petri dishes. The "dirty" inoculum was prepared by the addition of 13·3 ml of a 5% (w/v) suspension of dry yeast to 20 ml stock inoculum. The fungi were grown on Sabouraud agar for 10 d at 25°C. the stock inocula were prepared bye the same procedure as that used for the bacteria, except that the amount of live cells in the "clean" inoculum was ca. 2-5 x 10^7 /ml.

Yeast suspension

The 5% (dry w/v) yeast-suspesion was prescribed as the organic matter to be used in the test. In the original procedure, blocks of bakers' yeast were included, but in the present investigation these were replaced with dehydrated yeast. The 5% dry yeast was allowed to rehydrate in sterile water for 10 min, and the suspension was sterilized immediately by autoclaving (121°C for 20 min).

Disinfectants

A: Fenyl-fenol 45% (Norsk Medisinaldepot, Oslo), a 45% (w/w) solution of o-phenyl-phenol in a linseed oil soap; soap content 6·5%.

B: Fenyl-fenol 45% (Norsk Medisinaldepot, Oslo), the same 45% (w/w) solution of o-phenylphenol in a soya oil soap; soap content 3·5%.

C: A disinfectant from "Bayer", a solution of p-chloro-m-cresol and o-benzyl-p-chloro-phenol, with a total of 9·2% (w/w) phenols in a detergent system.

Method I – capacity-use-dilution test

Disinfectants A and B were used in the following concentration: 1·0, 0·5 and 0·25% (v/v). The pH in the respective solutions were: 11·0, 10·5, 10·3 for disinfectant A; and 11·3, 10·8, 10·6 for disinfectant B. disinfectant C had a pH between 7·1 and 6·6. the latter was used in the following concentrations (v/v): 2·0, 0·4 and 0·2%; they were later increased to 4·0, 2·0 and 0·4%. The dilutions were made in sterile tap water. To each 3 ml disinfectant solution (in 25 ml containers) 1 ml of respectively the test bacterium/saline suspension (series a) and the test bacterium/yeast suspension (series b) was added in three steps: after 0, 10, and 20 min. Samples for growth were transferred (one drop from a Pasteur pipette (1/30 ml)) after 8, 18 and 28 min, to each of two tubes containing HS-T broth; one drop was spread over the whole surface of blood agar in a Petri dish. Media with test samples of bacteria were incubated for 4 d at 37°C. In the case of C. albicans incubation was for 4 d, and for 10 d at 25°C with A. fumigatus.

The lowest disinfectant concentration which was considered safe for use was that which permitted at least two incremental yeas addition before a positive culture appeared.

Method II

Geometrical dilutions of each disinfectant were made in a double series in glass tubes each containing 1 ml test solution. The recommended use-dilution was in the highest concentration employed. A sample of the test bacterium/saline suspension (0·1 ml-3 drops from an adjusted Pasteur pipette) was added to each test tube in the first and a sample of bacterium/yeast suspension (0·1 ml) to each tube in the second series. After 8 min one platinum loop (4 mm. i.d.) full of the test solutions was transferred to 10 ml HS-T broth, and incubated at the same temperature and for the same time as described under Method I. All experiments were done at room temperature (ca. 22°C) and controls free from disinfectants were run parallel with the ordinary test series.

RESULTS AND DISCUSSION

Test resulting using Method I (Table 1) displayed no difference between the bactericidal and fungicidal effect of disinfectant A and B. they showed that a use-dilution concentration of 5·0% seems to give a very good safety margin.

TABLE 1

Capacity-use-dilutions in % (v/v) evaluated for three disinfectants under "clean" and "dirty" conditions*

Test organisms	A+		B+		C+	
	in saline	in yeast	in saline	in yeast	in saline	in yeast
Escherchia coli	0·5	1-0·5	0·5	1-0·5	0·4	2
Pseudomonas aeruginosa	<0·5	1	0·5	1	2	>2
Streptococcus faecalis	0·5	1	0·5	1	0·4	>2
Streptococcus aureus	0·5	>1	0·5	>1	2	>2
Proteus vulgaris	0·5	0·5	0·5	0·5	0·4	4
Candida albicans	1	1	0·5	1	<0·4	2
Aspergillus fumigatus	0·5	0·5	0·25	0·5	2	4

*The test was performed at 22°C. Results obtained from two runs.
+A, a 45% solution of o-phenylphenol in linseed oil soap; B, a 45% o-phenylphenol in soya oil soap; C, a solution of p-chloro-m-cresol and o-benzyl-p-chlorophenol in a detergent.

The "safe" use-dilution concentration in this experiment was evaluated to be 2·0%. The use-dilution concentration (2·0%) recommended for disinfectant C was insufficient for most of the test organisms. A concentration of 4% was required for P. vulgaris and A. fumigatus. The minimum germicidal concentration (MGC) of the disinfectants after 8 min exposure were evaluated from the resulting Method II (Table 2). Disinfectant A and B showed very similar MGC values for the different test strains. The most resistant of the organisms was S. aureus strain which required a MGC of 1·0%. Apart from S. aureus, the different test organisms displayed almost the same resistance to disinfectant C. when using the "dirty" conditions, the concentration needed to eliminate S. aureus had to be increased four times.

TABLE 2

Lowest germicidal concentration (MGC) in % (v/v) of three disinfectants at "clean" and "dirty" conditions in 8 min*

Test organisms	A+		B+		C+	
	in saline	in yeast	in saline	in yeast	in saline	in yeast
Escherchia coli	0·32	0·62	0·32	0·62	0·125	0·5
Pseudomonas aeruginosa	0·16	0·32	0·32	0·32	0·25	0·5
Streptococcus faecalis	0·16	0·16	0·16	0·32	0·125	0·25
Streptococcus aureus	0·62	0·62	0·62	0·62	0·25	1·0
Proteus vulgaris	0·16	0·16	0·16	0·16	0·25	0·5
Candida albicans	0·32	0·32	0·62	0·62	0·125	0·5
Aspergillus fumigatus	0·16	0·32	0·16	0·16	0·25	0·5

*The test was performed at 22°C. results obtained from two parallel runs.
+A, a 45% solution of o-phenylphenol in linseed oil soap; B, a 45% o-phenylphenol in soya oil soap; C, a solution of p-chloro-m-cresol and o-benzyl-p-chlorophenol in a detergent.

CONCLUSIONS

In the capacity-use-dilution test, a liquid recovery medium was used or alternatively a nutrient agar medium. Also used various inactivators in nutrient broth as recovery media. When testing the phenyl-phenol disinfectant they used a solution of 2% (v/v) Tween 80 and 1% (w/v) lecithin. In the present investigation HS-T broth was introduced as a recovery medium. This medium contains both Tween 80 and lecithin each of which is reported to inactivate both phenols and cresols. The HS-T broth is also reported to be a good recovery medium when bacteria have been exposed to stress.

The horse-blood agar was used because it contains native organic material (blood/serum) which to a certain extent inactivates the disinfectant. The phenyl-phenol disinfectant is a good bactericidal agent. Almost equal use-dilution concentration of disinfectant A and B (between 0·5- 1·0%) were evaluated for most of the test strains, which is much lower than the recommended use-dilution.

It was mentioned that this method is not always reproducible, but in the present investigation the same use-dilution concentration of disinfectant A was required both for E. coli and S. aureus. Fr disinfectant C, a use-dilution concentration of 4·0% was found to kill certain of the test organisms. However, the content of phenols in a 4·0% (v/v) solution of this disinfectant is lower than in a of 1·0% (v/v) solution of disinfectant A. the stronger effect in the lower phenol-containing disinfectant might be due to the p-chloro-m-cresol and o-benzyl-p-chlorophenol, and/or the lower pH value in the use-dilution concentration. The pH in the use-dilution of disinfectants A and B was ca. 11, whereas that of the disinfectant C was ca 7, and it is known that the phenols are more active in an acid milieu. The detergent system in disinfectant C might also contribute to its strong germicidal effect. The disinfectant displayed lower MGC values than

expected when comparing its effect with the capacity use-dilution concentrations evaluated for all the organism tested, excepting S, aureus. However, the disinfectant was much influenced by organic material.

When using the present test methods the two different soaps in the o-phenyl-phenol disinfectant did not display any influence in the exterminating capacity of this compound against the various micro-organism tested, and hence that recommended use-dilution can be the same for disinfectant B as it was for disinfectant A.

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