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Note

Comparison of Fungicidal Effects of Commercial Disinfectants at Concentrations Suggested for Practical Use

JI-CHENG HUANG[1,2], KOSUKE TAKATORI[1*], TOSHIKO OHTA[1,3], JUNKO KOSUGE[1], ATSUKO TAKAHASHI[4], AND SUSUMU KUMAGAI[5]

[1]National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya, Tokyo 158, Japan, [2]Insititute of Food Hygiene Inspection and Examination of Guang Dong Province, No.176, Xin Gang Xi Ru, Guang Zhou, China, [3]Sagami Women's University, 2-1-1, Bunkyo, Sagamihara, Kanagawa 228, Japan, [4]Food & Drug Safety Center, 729-5, Ochiai, Hadano, Kanagawa 257, Japan, and [5]National Institute of Health, 1-23-1, Toyama, Shinjuku, Tokyo 162, Japan

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Examination was made of the effects of nine commercial disinfectants in short term application at concentrations recommended for practical use against 34 fungi. The effects of a yeast extract comprised of various organic substances on the efficacy of the disinfectants were also studied. Ethanol (EtOH, 70%, v/v) was found the most effective disinfectant and not influenced by yeast extract at a concentration of 5% (w/v). Oxidizing potential liquid (OPL) with 55 μg/ml as the active chlorine concentration, iodine (IA) at 45 μg/ml and sodium hypochloride (NaOCl) at 200 μg/ml all showed strong fungicidal effects, which were, however, significantly diminished by yeast extract. Didecyldimethylammonium chloride (DDAC) at 100 μg/ml, benzalkonium chloride compounds (BACC) at 285 μg/ml and benzalkonium chloride (PBKC) at 200 μg/ml had a smaller fungicidal effects but there effects were less altered by the extract. [Mono-bis (trimethylammonium methylene chloride)]-alkyl (C_{9-15}) toluene (MBAT) at 100 μg/ml and chlorhexidine digluconate (CHD) at 200 μg/ml, showed the smallest fungicidal effects.

Key words : Disinfectant/Fungi/Fungicidal effect.

Effective disinfectants are required for sterilization and foodstuff sanitation and must be applied properly. Ethanol, guanidine, chlorine, iodine and quaternary ammonium compounds are generally recommended for sterilization since they have long proven to be highly effective as disinfectants (Dychdala, 1977; Ishii, 1993). Oxidizing potential liquid (OPL) has also recently come into wide use at hospitals and food manufacturing establishments owing to its remarkable microbicidal activity and low toxicity (Okubo, 1995; Shimizu and Ukon, 1994). The antibactericidal activity of these disinfectants has been extensively studied and found to be quite satisfactory for eliminating bacteria through proper application (Dychdala, 1997; Shibasaki, 1985). Methods for disinfectant evaluation, however are concerned primarily with the determination of the minimum inhibitory concentration (MIC) rather than that of the minimum bactericidal concentration (MBC), and the effects of disinfectants have been studied for only a limited number of fungal species present in the environment. The MIC and MBC of fungicides have been noted to differ significantly (Colombo, et al., 1994). In food manufacturing, disinfectants must kill microbes to be effective. The contact time of disinfectants for sterilization is generally quite short (Dychdala, 1977) and thus concentrations actually used are always higher than the MIC.

Corresponding author. Tel : +81-3-3700-9538, Fax : +81-3-3700-9538.

TABLE 1. Evaluation of fungicidal effects of disinfectants.

Fungus	Disinfectant[a]								
	MBAT 100 μg/ml	BACC 285 μg/ml	DDAC 100 μg/ml	PBKC 200 μg/ml	OPL 55 μg/ml	NaOCl 200 μg/ml	IA 45 μg/ml	EtOH 70 %	CHD 200 μg/ml
Aureobasidium pullubans	++++[b]	++++	++++	++++	++++	++++	++++	++++	++++
Byssochlamys nivea	++++	++++	++++	++++	++++	++++	++++	++++	++++
Eurotium chevalieri	++++	++++	++++	++++	++++	++++	++++	++++	++++
Fusarium moniloforme	++++	++++	++++	++++	++++	++++	++++	++++	++++
Fusarium oxysporum	++++	++++	++++	++++	++++	++++	++++	++++	++++
Fusarium solani	++++	++++	++++	++++	++++	++++	++++	++++	++++
Paecilomyces variotii	++++	++++	++++	++++	++++	++++	++++	++++	++++
Phoma sp.	++++	++++	++++	++++	++++	++++	++++	++++	++++
Rhodotorula rubra	++++	++++	++++	++++	++++	++++	++++	++++	++++
Saccharomyces cerevisiae	++++	++++	++++	++++	++++	++++	++++	++++	++++
Wallemia sebi	++++	++++	++++	++++	++++	++++	++++	++++	++++
Trichosporon cutaneum	++++	++++	++++	++++	++++	++++	++++	++++	+++
Cladosporium cladosporioides	+++	++++	++++	++++	++++	++++	++++	++++	+++
Alternaria alternata	++++	+++	+++	++++	++++	++	++++	++++	+++
Neosartorya fischeri	+++	+++	+++	++++	++++	++++	++++	++++	++
Aspergillus ochraceus	++	++++	++++	+++	+++	++++	++++	++++	+++
Aspergillus restrictus	++	+++	++++	++++	++++	++++	++++	++++	+++
Aspergillus niger	++	++	++	++++	+++	++++	++++	++++	++
Aspergillus versicolor	++	+++	+++	++++	++++	++++	++	++++	++
Penicillium cyclopium	++	+++	+++	++	++++	++++	+++	+++	++
Penicillium citrinum	++	+++	+++	+++	++++	++++	++	++++	++
Penicillium citreo-viride	+++	+++	+++	+++	++++	++++	++	++++	++
Penicillium expansum	++	++	+++	++	++++	+++	++++	++++	++
Trichoderma sp.	+	++	++++	++	++++	++++	++	++++	−
Aspergillus clavatus	−	++	+++	++	+++	++++	++	++	++
Aspergillus fumigatus	−	+++	++++	++++	+++	+++	+++	++++	−
Aspergillus flavus	−	−	−	++	+++	++++	+++	++++	−
Aspergillus parasiticus	−	+	+++	++	+++	+++	++++	++++	−
Penicillium islandicum	−	−	−	−	+++	+++	+++	++++	−
Rhizopus stolonifer	−	+	−	+	+++	++	++++	++++	−
Mucor sp.	−	−	−	−	++++	++++	++++	++++	−
Emericella nidulans	−	++	+++	++	++	+++	++++	++++	−
Arthrinium sp.	−	−	−	−	++	++	+++	++++	−
Chaetomium globosum	−	−	−	−	−	−	−	++	−

[a]See text for abbreviations.
[b]Fungicidal effect was evaluated based on the time required to kill the fungi, as follows : ++++, ≦ 0.5min ; +++, 1~2 min ; ++, 5~10min ; +, 15~30min ; −, ≧ 60min.

Furthermore, it should be clarified whether disinfectants are effective for fungal disposal under the same conditions as for treating bacteria, especially in food manufacturing. Data presently available on the use of disinfectants should serve as a basis on which disinfectants can be properly selected according to purpose.

Thirty four fungal specimens (Table 1) were obtained from the Microbiology Department of the National Institute of Health Sciences, Tokyo, Japan. They had been taken from foods such as corn, wheat, beans, rice and sweets, as well as from food manufacturing equipment and the environment.

Spore suspensions supplemented with 0.05 % (w/v) Tween 80 - saline were prepared by rubbing the surfaces of fungal colonies onto potato dextrose agar (PDA) or M40Y (malt-sucrose-yeast-agar) slants which were incubated at 25℃ for 7d. Spores were counted using a haemocytometer and spore density was adjusted to 1.0×10^6/ml in all final suspensions.

Disinfectant concentrations in this study were those recommended for practical use and, along with pertinent data, are given as follows: EtOH, Wako Pure Chemical Co., 70 % (v/v); OPL, Cleanup, Nichiden Co., 55 μg/ml, ORP 1170mV, pH2.4; IA, Wako Pure Chemical Co., 45 μg/ml; NaOCl, Wako Pure Chemical Co., 200 μg/ml; DDAC, Wako Pure Chemical Co., 100 μg/ml; PBKC, Wako Pure Chemical Co., 200 μg/ml; BACC, Osvan, Nihon Pharmaceutical Co., 285 μg/ml; MBAT, Pacoma, Eizai Co., 100 μg/ml and CHD, Hibitane, Sumitomo Pharmaceutical Co., 200 μg/ml.

All solutions were diluted with sterile distilled water prior to use. The disinfectants were evaluated for efficacy during short term application. Their solutions were adjusted to the test concentrations and introduced into test tubes at 2 ml each. A one tenth ml aliquot of spore suspension was added to each tube kept at 25℃ and the system was vortexed. One tenth ml was taken from each tube after each incubation at 25℃ for 0.5, 1, 2, 5, 10, 15, 20, 30, and 60 min and diluted immediately with 2 ml glucose peptone broth and then incubated at 25℃ for 7 d. The spores were again counted as above. A yeast extract as a mixture of organic constituents was examined for its effect on disinfectant activity at concentrations of 0.1, 1 and 5 % (w/v). Five fungal specimens that would be readily affected by disinfectants in the absence of the organic constituents were used for this study. All experiments were conducted in duplicate.

Fungicidal effects of the disinfectants examined are summarized in Table 1. EtOH was found the most effective, inactivating all fungi within 10 min. 31 specimens were completely inactivated by EtOH within 0.5 min. IA, OPL and NaOCl were effective against 33 species within 10 min. Only one species was resistant to all these disinfectants. PBKC, DDAC and BACC showed high fungicidal activity, inactivating 80 % of 34 test fungi within 10 min. MBAT and CHD had the smallest effect on the fungi, with no activity against as many as 10 specimens. The order of disinfectant efficacy was found to be as follows: EtOH > IA ≅ OPL ≅ NaOCl > PBKC > DDAC ≅ BACC > MBAT ≅ CHD.

Fungal resistance differed according to the disinfectant (Table 1). *Chaetomium globosum* was most resistant to all disinfectants and could grow even after 60 min of EtOH treatment. *Aspergillus flavus, A. fumigatus, A. parasiticus, A. clavatus, Emericella nidulans, Penicillium islandicum, Arthrinium* sp., *Mucor* sp., *Rhizopus stolonifer, Trichoderma* sp. were capable of resisting MBAT, CHD, BACC, DDAC and PBKC. All other fungal specimens were very susceptible to the disinfectants, most being killed within 5 min.

The choice of disinfectant should thus be made according to the fungus under consideration. The effects of yeast extract for inhibiting disinfectant activity are

TABLE 2. Effects of yeast extract on the disinfectant activity.

Fungus	Yeast extract (%, w/v)	Disinfectant[a]								
		MBAT 100 μg/ml	BACC 285 μg/ml	DDAC 100 μg/ml	PBKC 200 μg/ml	OPL 55 μg/ml	NaOCl 200 μg/ml	IA 45 μg/ml	EtOH 70 %	CHD 200 μg/ml
Fusarium solani	0	++++[b]	++++	++++	++++	++++	++++	++++	++++	++++
	0.1	+++	++++	+++	++++	+	++	+++	++++	+++
	1.0	++	++	++	++	−	−	−	++++	−
	5.0	++	+	+	+	−	−	−	++++	−
Aureobasidium pullubans	0	++++	++++	++++	++++	++++	++++	++++	++++	++++
	0.1	+++	+++	+++	+++	+	−	+++	++++	+++
	1.0	++	++	++	++	−	−	−	++++	++
	5.0	+	−	−	−	−	−	−	++++	+
Cladosporium cladosporioides	0	++++	++++	++++	++++	++++	++++	++++	++++	++++
	0.1	+++	++++	++++	++++	+	++	+	++++	+++
	1.0	++	++	++	++	−	+	−	++++	++
	5.0	++	+	+	++	−	−	−	++++	−
Rhodotorula rubra	0	++++	++++	++++	++++	++++	++++	++++	++++	++++
	0.1	++	++	+++	++++	−	−	+++	++++	+++
	1.0	+	−	++	++	−	−	−	++++	−
	5.0	−	−	+	−	−	−	−	++++	−
Aspergillus niger	0	++++	++++	++++	++++	++++	++++	++++	++++	++++
	0.1	+	++	++	+++	−	−	+	++++	+
	1.0	−	−	−	−	−	−	−	++++	−
	5.0	−	−	−	−	−	−	−	++++	−

[a]See text for abbreviations of disinfectants.
[b]See footnote to Table 1 for symbols.

shown in Table 2. Only EtOH was not affected by the extract. OPL, IA and NaOCl at an extract concentration of more than 1 % (w/v) were affected the most. Five fungal specimens were virtually unaffected by the disinfectants in the presence of the extract. The degree of disinfectant inhibition followed the order, PBKC, DDAC, BACC, MBAT and CHD, and was greater than any of those for OPL, IA and NaOCl, in this order. The activity of EtOH was not affected by the yeast extract.

EtOH is shown by the present results to exert the greatest fungicidal activity, which is not diminished by any organic substance. EtOH is particularly suitable as a disinfectant since it is colorless and readily evaporates. It is inexpensive and can be easily obtained, EtOH has thus come into wide use as a disinfectant, though it is ineffective toward nearly all bacterial spores (Dychdala, 1977).

OPL, IA and NaOCl showed remarkable fungicidal effect which was greatly diminished by the yeast extract. Organic substances may function to remove active chlorine and iodine, thus eliminating microbicidal activity. The presence of such substances should thus be confirmed prior to disinfectant application.

OPL is prepared by the electrolysis of saline. Its chlorine constituent is low (55 μg/ml), but ORP is high (ORP 1170 mV, pH2.4). This feature serves to augment the permeability of the cell membrance and stimulate the antimicrobial activity of active chlorine. The low pH enhances the action of chlorine. OPL undergoes reduction into water and NaCl, so that there are no toxic residues following its use. OPL is standardly used at hospitals and in food processing. Its high microbiocidal effect is greatly reduced at low yeast extract concentrations. Equipment to be disinfected should thus be washed prior to OPL application.

The fungicidal effects of EtOH exceeded those of OPL, IA and NaOCl and these effects were not significantly affected by the yeast extract and have been shown relatively stable in solution (Ishii, 1993 ; Shibasaki, 1985).

The low fungicidal effect of CHD at the concentration examined is consistent with its antibacterial activity as determined in a previous study (Kikuchi et al., 1995 ; Sagripanti et al., 1996). EtOH, OPL, NaOCl and IA were shown to be effective fungicides at the concentrations studied; PBKC, BACC and DDAC should be applied at higher concentrations to effectively dispose of certain resistant fungi and this is particularly so for MBAT and CHD.

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