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RESEARCH ARTICLE

THE EFFECTS OF QUATERNARY AMMONIUM COMPOUNDS BASED DISINFECTANT BY IMAGO & GETTER, ON SOME IMPORTANT FOODBORNE PATHOGENS

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ABSTRACT

Antimicrobial disinfectants are widely used in different food processing industry across the world. In attention to extent uses of disinfectants, it is necessary to evaluate and validate the efficacy of disinfectants and employing the minimum effective dosages. In this research the antibacterial influences of a common disinfectant solution used in food industry, quaternary ammonium compound were evaluated on six important food borne pathogens including two Gram positive (*Staphylococcusaureus* and *Bacillus subtilis*), two Gram negative bacteria (*Escherichiacoli* and *Pseudomonas aeruginosa*) and two fungus (*Candida albican to Candida albicans*). According to the obtained results, Bacillus subtilis was most resilient whereas the other entire test organisms were susceptible. In attention to obtained results, the used disinfectant has good antibacterial and anti fungal effects.

INTRODUCTION

Food industry is most successful and growing industry in urban world but they are prone to exposure of different microbes as food provides sourceand environment for microbial growth (1). The microorganisms in the industry have the potential to transmit from equipment surface to food and processing area (2). Thus the equipment surfaces which are used for food handling and processing are categorised as major sources of microbial contamination. There are numerous cases of foodborne illness by the presence of pathogenic microorganisms on fresh products. It is very important to eliminate the microbes which are dwelling in industrial environment. At food industry, the most effective way of controlling levels of pathogenic microorganisms is regular cleaning and disinfection procedures (3, 4). Cleaning and disinfection assures eradication of microbes as well as their source of contamination. The cleaning procedure is done to remove different soils, pollutants and remove surface contamination by the use of detergents which are generally not antimicrobial agents. Once proper cleaning is done, the area is subjected to disinfection forreduction ofviable remaining organisms (4). There are different types of disinfectants that are popularly used at food industry for food utensils, area, surface and environment. Disinfectant such as alcohol based products, hypochloric solutions (sodium hypochlorite), peracetic acid, and quaternary ammonium compounds (QACs) and synthetic antimicrobial agent with broad spectrum antimicrobial (4-6).

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The QACs are widely used for cleaning and sanitization at dairy industry, food storage tanks, catering industry and fisheries. These are considered effective and safe as these are non-corrosive and possess broader anti-microbial spectrum (7). QAC based disinfectants are nontoxic, non-tainting and odor free at use dilutions and compatible with nonionic, ampholytic and cationic surface active agents (8). QACs biocides are surfactants which are cationic in nature contains one quaternary nitrogen associated with at least one major hydrophobic substitute for the use of disinfectant (7)(9). The different alkyl chain of QACsshows effective bactericidal, fungicidal, virucidal(non-enveloped) and tuberculocidal activities without introducing any side effect. QACs irreversibly bind to the phospholipids and proteins of the membrane, thereby allowing permeability of the cells, which leads to its degeneration. The antimicrobial activity of quaternary ammonium with an alkyl chain is related to lipophilia and peaks between C12 and C16 (10). In the present study three QAC based products of Imago & Getter namely Imagrad IG PRO 401, Imagard IL 15 and Imagard SF 25 was used against some food pathogenic microorganisms and to evaluate the bacterial and fungal effectiveness against some important foodborne Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) pathogens as well as fungus (*Candida albican* and *Aspergillusbrasiliensis*).

MATERIALS AND METHODS

Preparation of Disinfectant concentration: The disinfectants were obtained from Imago & Getter, India.Imagard IG PRO401 was diluted as 4ml in 1 litre of Deionised water to

obtain 0.4% v/v, Imagard IL 15 was diluted as 15ml in 1 litre of Deionised water to obtain 1.5% v/v and Imagard SF 25 was diluted as 25ml in 1 litre of Deionised water to obtain 2.5% v/v.

Test Organism and its Suspension

Standard strains of the test organisms of *Staphylococcus aureus (ATCC 6538), Bacillus subtilis (ATCC 6633), Escherichia coli (ATCC 11229), Pseudomonas aeruginosa (ATCC 9027) Candida albicans (ATCC 10231)* and *Aspergillusbrasiliensis (ATCC 16404)* (11) were obtained from National Collection of Industrial Microorganisms (NCIM), Pune, India. Suspension of each of the test organisms was made by collecting a loopful of colony from each plate and inoculating in sterile peptone water. The tubes of the subcultured organisms were incubated for bacteria at 30 - 35°C for 24 to 48 hours and for fungal at 20 - 25°C for 3-5 days. Adjust the cell density to approximately 1.0 x 10^7 CFU/mlusing the diluent. For counting of fungal test suspension prepare 1.0 - 1.5 x 10^7 CFU/ml.

Test procedure

This testing was done according to European standards (EN) Guideline for the suspension Test (12). The simple suspension test EN 1040:1997 and qualitative suspension test(phase 2, part 1) EN 1650:1997 was conducted for the bactericidal and fungicidal/yeasticidal activity respectively. The disinfectants were diluted are per concentration recommendation (at room temperature) and the contact time for all the disinfectant was at 2 min, 5min & 10min.

done, filter 0.1ml of each culture dilution and rinse the membrane with 1 x 100 ml of the sterile 0.1% peptone water through 0.45 µ membrane filters. After rinsing, place each membrane filter on the surfaces of individual pre-incubated sterile Tryptone Soya Agar plates. Similarly repeat the procedure for all the inoculums for respective time. Incubate the plates at 30-35°C for 3 days for bacteria and at 20-25° for 5days for yeast and fungi. Keep one contact plate of Tryptone Soya Agar plate as negative control. For positive control take the sample of the culture surfaces of positive control coupon of each type of surfaces with sterile swab. Transfer the swab to 10 mL of Dey/Engley broth. Vortex the tubes containing swab for about 30 seconds (13). Compare the culture plates after 2 min, 5 min and 10 min contact time for disinfectant and calculate the Log reduction using the formula:

Final Log Reduction = Log (Initial Count) – Log (Final count)

Acceptance Criteria

Since microorganisms vary in their susceptibility to disinfection procedures, European standard ENfor suspension test recommends an expectation of 4 log10 of reduction for vegetative bacteria and ≥3 log10 of reduction for fungi/yeast (14).

RESULTS

According to the obtained results it is observed the entire Imagard products from Imago & Getter are giving log 4 reduction.

Table 1. The bactericidal activity of Imagard IL 15at 1.5% concentration for the gram positive and gram negative bacteria

Product identification	Test organism	Exposure time	Count of test organism			Anti-microbial activity	
			Initial count	After exposure		Log reduction	Percentage reduction
				CFU/ml	Log		
Imagard IL 15	*S. aureus*	2 mins.	1.75×10^5 L = 5.24	< 10	< 1	>4.24	>99.99
		5 mins.		< 10	< 1	>4.24	>99.99
		10 mins.		< 10	< 1	>4.24	>99.99
	B. subtilis	2 mins.	1.93×10^5 L = 5.28	< 10	< 1	>4.28	>99.99
		5 mins.		< 10	< 1	>4.28	>99.99
		10 mins.		< 10	< 1	>4.28	>99.99
	E. coli	2 mins.	1.80×10^5 L = 5.25	< 10	< 1	>4.25	>99.99
		5 mins.		< 10	< 1	>4.25	>99.99
		10 mins.		< 10	< 1	>4.25	>99.99
	P. aeruginosa	2 mins	1.56×10^5 L = 5.19	< 10	< 1	>4.19	>99.99
		5 mins.		< 10	< 1	>4.19	>99.99
		10 mins.		< 10	< 1	>4.19	>99.99

Table 2. The fungicidal activity of Imagard IL 15 at 1.5% concentrationfor yeast and mold

Product identification	Test organism	Exposure time	Count of test organism			Anti-microbial activity	
			Initial count	After exposure		Log reduction	Percentage reduction
				CFU/ml	Log		
Imagard IL 15	C.albicans	2 mins.	1.90×10^5 L= 5.27	< 10	< 1	> 4.27	>99.99
		5 mins.		< 10	< 1	> 4.27	>99.99
		10 mins.		< 10	< 1	> 4.27	>99.99
	A. brasilliensis	2 mins.	1.10×10^5 L= 5.04	< 10	< 1	> 4.04	>99.99
		5 mins.		< 10	< 1	> 4.04	>99.99
		10 mins.		< 10	< 1	> 4.04	>99.99

Add 0.1 ml of the prepared challenge inoculum (containing around 10,000 to 100,000 CFU) of Bacillus subtilis in a sterile test tube containing 10 ml of sterile Dey/Engley broth. Dilute this solution using 10-fold dilution method from $10^{-1}$to 10^{-4} using sterile 9 mL Dey/Engley broth. After the dilution is

Therefore, this indicates that all the disinfectants have excellent antimicrobial efficacy at recommended concentration and time. The use of all the mentioned disinfectants may be means to reduce the contamination caused by the test microorganisms.

Table 3. The bactericidal activity of Imagard IG PRO 401 at 0.4% concentration for the gram positive and gram negative bacteria

Product identification	Test organism	Exposure time	Count of test organism			Anti-microbial activity	
			Initial count	After exposure		Log reduction	Percentage reduction
				CFU/ml	Log		
Imagard IG PRO 401	*S. aureus*	2 mins.	1.55×10^5 L = 5.19	< 10	< 1	>4.19	>99.99
		5 mins.		< 10	< 1	>4.19	>99.99
		10 mins.		< 10	< 1	>4.19	>99.99
	B. subtilis	2 mins.	2.75×10^5 L = 5.43	< 10	< 1	>4.43	>99.99
		5 mins.		< 10	< 1	>4.43	>99.99
		10 mins.		< 10	< 1	>4.43	>99.99
	E. coli	2 mins.	1.86 x 105 L = 5.26	< 10	< 1	>4.26	>99.99
		5 mins.		< 10	< 1	>4.26	>99.99
		10 mins.		< 10	< 1	>4.26	>99.99
	P. aeruginosa	2 mins	2.06 x 05 L = 5.31	< 10	< 1	>4.31	>99.99
		5 mins.		< 10	< 1	>4.31	>99.99
		10 mins.		< 10	< 1	>4.31	>99.99

Table 4. The fungicidal activity of Imagard IG PRO 401 at 0.4% concentration for yeast and mold

Product identification	Test organism	Exposure time	Count of test organism			Anti-microbial activity	
			Initial count	After exposure		Log reduction	Percentage reduction
				CFU/ml	Log		
Imagard IG PRO 401	*C.albicans*	2 mins.	2.10×10^5 L=5.32	< 10	< 1	> 4.32	>99.99
		5 mins.		< 10	< 1	> 4.32	>99.99
		10mins.		< 10	< 1	> 4.32	>99.99
	A. brasilliensis	2 mins.	1.92×10^5 L=5.28	< 10	< 1	> 4.28	>99.99
		5 mins.		< 10	< 1	> 4.28	>99.99
		10mins.		< 10	< 1	> 4.28	>99.99

Table 5. The bactericidal activity of Imagard SF 25 at 2.5% concentration for the gram positive and gram negative bacteria

Product identification	Test organism	Exposure time	Count of test organism			Anti-microbial activity	
			Initial count	After exposure		Log reduction	Percentage reduction
				CFU/ml	Log		
Imagard SF 25	*S. aureus*	2 mins.	1.26×10^5 L = 5.10	< 10	< 1	>4.10	>99.99
		5 mins.		< 10	< 1	>4.10	>99.99
		10 mins.		< 10	< 1	>4.10	>99.99
	B. subtilis	2 mins.	1.75×10^5 L = 5.24	< 10	< 1	>4.24	>99.99
		5 mins.		< 10	< 1	>4.24	>99.99
		10 mins.		< 10	< 1	>4.24	>99.99
	E. coli	2 mins.	3.40×10^5 L = 5.53	< 10	< 1	>4.53	>99.99
		5 mins.		< 10	< 1	>4.53	>99.99
		10 mins.		< 10	< 1	>4.53	>99.99
	P. aeruginosa	2 mins	2.16×10^5 L = 5.33	< 10	< 1	>4.33	>99.99
		5 mins.		< 10	< 1	>4.33	>99.99
		10 mins.		< 10	< 1	>4.33	>99.99

Table 6. The fungicidal activity of Imagard SF 25 at 2.5% concentration for yeast and mold

Product identification	Test organism	Exposure time	Count of test organism			Anti-microbial activity	
			Initial count	After exposure		Log reduction	Percentage reduction
				CFU/ml	Log		
Imagard SF 25	*C. albicans*	2 mins.	1.10×10^5 L= 5.70	< 10	< 1	> 4.70	>99.99
		5 mins.		< 10	< 1	> 4.70	>99.99
		10 mins.		< 10	< 1	> 4.70	>99.99
	A. brasilliensis	2 mins.	1.08×10^5 L= 5.03	< 10	< 1	> 4.03	>99.99
		5 mins.		< 10	< 1	> 4.03	>99.99
		10 mins.		< 10	< 1	> 4.03	>99.99

The most susceptible organism of the entire test organism subjected to Imagard IL 15, Imagard IG PRO 401 and Imagard SF 25 was *S. aureus* followed by *E. coli.*

DISCUSSION

In present study, QAC based disinfectant by Imago & Getter, showed strong bactericidal and fungicidal activity. The result shows that *Candida albican*and *Bacillus subtilis*were the resilient as compared to other gram negative bacteria and mold. The study shows that other Gram positive bacteria were more sensitive than Gram negative bacteria, except *Bacillus subtilis*because of its ability to spore formation. Similar to our study, there are reports proving that spore forming bacteria such as *Bacillus cereus* are considered to be resistant to QAC (15) whereas in other studies Gram positive bacteria are considered more susceptible than Gram negative (16). In Gram negative bacteria, the resistance mechanisms are more complicated due to presence of an inner and an outer membrane. The latter membrane has a clear role in modulating the accessibility of a cell to preservatives and other small molecules; the lipopolysaccharide layer is of crucial importance in this respect (17, 18). Although biocides can generally be regarded to act non-specific and or multifactorial

at use concentrations (19), resistance to biocides is of interest for the medical area, especially if a cross resistance to antibiotics is observed, as reported for *Staphylococcus* spp. isolated from the food industry and also for *Pseudomonas aeruginosa*for QACs(20).

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