Journal of Bacteriology and Parasitology

Hualpa and Ludeña, J Bacteriol Parasitol 2015, 6:3
DOI: 10.4172/2155-9597.1000232

Research Article Open Access

Evaluation Germicidal of Disinfectants on *Staphylococcus aureus* and *Escherichia coli*

Diana Hualpa[1*] **and Fanny Ludeña**[2]

[1]*Universidad Técnica Particular de Loja, Department of Agricultural and Food Sciences, San Cayetano Alto, Loja, Ecuador*

[2]*University Agraria La Molina, Graduate School Av La Molina s/ n. La Molina, Lima, Peru*

[*]**Corresponding author:** Diana Hualpa, Universidad Técnica Particular de Loja, Department of Agricultural and Food Sciences, San Cayetano Alto, Loja 110042, Ecuador, Tel: 59373701444; E-mail: dihualpa@utpl.edu.ec

Received date: March 27, 2015; **Accepted date:** June 28, 2015; **Published date:** June 30, 2015

Abstract

The germicide efficiency of nine common disinfectants used in the food industry was evaluated against *Sthaphylococcus aureus* (ATCC 6538) and *Escherichia coli*. (ATCC 8739). Quaternary ammonium, chlorine and peracetic acid combined with hydrogen peroxide were used for disinfection of inert surfaces in contact with the product. While for disinfection of living surfaces (hands), ethyl alcohol, surfactants and alcohols with quaternary ammonium compounds in its composition were considered and glutaric aldehyde, benzyl-C12-C16-alkyl-dimetityl-ammoniumchloride and phosphate for footbaths. The disinfectants were applied at different concentrations (0.3-3%) and time of contact; the germicide efficiency was evaluated for each disinfectant by the plate count of survivor microorganism's method. A neutralizer solution was used to inactivate the disinfectant and to perform correctly the count. Results showed that in the case of inert contact surfaces there was not significant differences in the germicidal efficiency among disinfectants ($p>0.05$). In the case of living surfaces (hands) the best disinfectant ($p<0.05$) was alcohol including in its composition quaternary ammonium compound at 1.7% with an exposure time of 2 minutes. For footbaths the disinfection treatment corresponded to benzyl-C12-C16-alkyl-dimetityl-ammoniumchloride at 0.3% ($p<0.05$) with an exposure time of 15 minutes shown the best results. Disinfectants containing quaternary ammonium compounds are effective against bacteria of *Staphylococcus aureus* and *Escherichia coli*.

Keywords: Evaluation germicidal; Contact surfaces; Hand surfaces; Footbaths; Disinfectants

Introduction

Cleaning and disinfecting are procedures to control the factors related to the asepsis in food processing plants. Equipment and instruments can lead to contamination and be vehicles of transmission of microorganisms that may cause changes in processed foods. These objects should be thoroughly cleaned and then disinfect to prevent cross-contamination with microorganisms. Proper cleaning and disinfection, along with hand washing is one the most effective measures to prevent contamination.

"Sanitizing is important because it helps reduce any of the microbes that may be left after the cleaning" says Martin Bucknavage, senior food safety extension associate at Penn State College of Agriculture, University Park, Pa. "the cleaning is going to remove any of the solids and any of the materials from the food manufacturing line or the food process. The sanitizing comes in and removes any of the microbes that might be there" [1].

It is very important not only choose the appropriate disinfectant if not show that the concentrations, time, temperature and physical action that is used is efficient and profitable for those who use them. This research demonstrated experimentally the effectiveness of these disinfectants. Also obtain security in the process of disinfection using a methodology of a standardized procedure.

Entis in 2002 [2] mentions that the disinfectant function is to destroy microorganisms and prevent the spread of these. A disinfectant is a biocide that destroys the growth of microorganisms on surfaces and inanimate objects [3].

Puig [4] states that disinfection is the destruction of microorganisms, applied on clean surfaces so as to reduce the number of microorganisms to a level that will not lead to harmful contamination of food in contact with surfaces. The disinfection as a final stage of a sanitation program is designed to remove waste products and foreign bodies while reducing the level of pathogenic microorganisms and to alter, to ensure both the quality and safety of food [5].

The organic material present, is able to reduce the ability of the biocide disinfectant due to its diluting effect [6]. Troya [7] mentions that disinfectants are classified according to the agent, which is responsible for destroying microorganisms and the action spectra have different properties.

The mechanisms of action of biocides, together with the factors that influence its activity, has become a key feature for the best use of biocidal formulations and control the emergence of resistant organisms [8]. It is considered that the active ingredients of the disinfectants are generally products that may contain one or more actives principles [9].

Antimicrobial agents can affect cells very differently. At high concentrations, some can precipitate proteins (clotting), may break the cell membrane or cause antagonism chemical to interfere with enzymatic reactions or removing their free sulfhydryl group [10].

Salas [8] states that the microbial activity of most disinfectants can be altered due to several factors such as concentration, pH, temperature, organic load and exposure time.

The surfaces hygiene affects the quality and safety of the food product [8]. Conditionally equipment and the environment should be designed hygienically (avoiding cracks or dead space) for an effective cleaning and disinfection (sanitation) is the fundamental control to contamination of these surfaces [8,11].

Also Wildbrett [9] mentions that the disinfection of "living surfaces" (hands) should always be considered the similarity in the structural constitution of these germs and vehicles.

Determining the germicidal efficacy is often carried out in suspension tests. This type of test determines the concentration of disinfectant which shows a definite log reduction in the number of microorganisms at a given time. In practice, meanwhile, the microorganisms are subjected to disinfection of surfaces in food production and that remains after cleaning, are commonly of the surface [8].

The microorganisms that are subject to the disinfection of surfaces in food production are those that remain after cleaning, and are attached to the surface [8].

Although it is recognized that microorganisms may be more resistant to disinfectants when are once adhered to the surface, suspension testing methods are still used as standards for evaluating disinfectants in food hygiene [8]

The objective of this research was to evaluate efficiency germicide of nine disinfectants to minimize risks and increase microbiological safety of products made in Ecolac Dairy plant.

Materials and Methods

Preparation of disinfectants

Disinfectants Tradename	Keys	Active Principle	Use
Saniquat	A	Quaternary ammonium	Inert surfaces
Rimadet-sr-300	B	Chlorine	Inert surfaces
Weicoper	C	Peracetic acid combined with hydrogen peroxide	Inert surfaces
Dr Clean	D	Ethyl alcohol	Living surfaces (hands)
Hand-des	E	Surfactants (triclosan)	Living surfaces (hands)
Klinosept-derm	F	Alcohols with quaternary ammonium compounds	Living surfaces (hands)
Rimasan-aq	G	Glutaric aldehyde	Footbaths
Weiquat	H	Benzyl-c12-c16-alkyl-dimetithyl-ammoniumchloride	Footbaths
Rimasan-v	I	Phosphate	Footbaths

Table 1: Disinfectants used to evaluate the efficiency germicidal.

The disinfectants evaluated are specified in Table 1, the solutions were prepared in distilled water (0.3 to 3%). Concentration used for each of the disinfectants corresponds to the recommended by the manufacturer to achieve one germicidal efficiency of 99.999% with reference to the study of Lopez et al. [12].

Preparation of bacterial inoculum

The bacterial inoculum was prepared with strains of *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739 developed individually on nutrient agar at a temperature of 35°C for 24 hours with concentrations of 107 CFU/ml [12]. Activation of the bacterial strains was performed in nutrient broth, incubated at 35°C by two hours and was adjusted la population n through scales turbidity of Mc Farland.

Preparation of the solution of inactivation

For all tests, after of contact time with the disinfectant is used one solution of inactivation of germicidal effect, to make counts surviving microorganisms, the same was prepared: 6 g/l lecithin, 60 ml/L Tween 80, 7.2 g/L bovine albumin, and 10 mL/L 0.25 M dihydrogen phosphate buffer [13].

Preparation of suspension to evaluation of footbaths and hand surfaces

The evaluation the efficiency germicidal was performed according Aarnisalo et al. [13] which consists in placing a bacterial suspension of *Staphylococcus aureus* and *Escherichia coli* in a 0.85% saline solution, then taking 2 ml of the same and was mixed with 2 ml solution of bovine albumin at 20°C for a period 2 minutes.

This solution containing the microorganisms under study are taken 1 ml and was added to respective tubes containing 24 ml of disinfectant solution and 24 ml saline acting as a control. Is left to act by the time set for test in footbaths and living surfaces (hand).

After of time action 0.5 ml was added in 4.5 ml of the suspension of inactivation and allowed to stabilize for 5 minutes. After this time we proceed to perform the respective dilutions and was seeded in triplicate in Petri dishes with nutrient agar and incubated at 35°C for 24 to 48 hours. The plate count of survivor microorganisms was performed considering the interval of 25 to 250 colonies CFU/ml according to FDA [14].

Preparation of the suspension to inert surfaces

To evaluate the germicidal efficiency on inert surfaces was proceeded to work according to Aarnisalo et al. [13] which involved taking the strains of *Escherichia coli* and *Staphylococcus aureus* activated in 2 ml of saline was allowed to act for 2 minutes after this time was put in stainless steel discs 12 mm in diameter previously disinfected with alcohol 70% and was sterilized for 15 minutes at 121°C and 15 PSI, incubated at 30°C for 24 ± 2 hours, then exposed disks in 2 ml each disinfectant and in the control and at the set times. The discs were immersed in the solution of inactivation to interrupt the effect of the disinfectant for 5 minutes.

From the solution of inactivation the respective dilutions were performed and plated on nutrient agar in triplicate and incubated at 30°C for 24 to 48 hours. The plate counts were made taking into

consideration the interval between 25 and 250 colonies CFU/ml according to FDA [14].

After of Microbial count was proceeded to calculate the efficiency germicidal of each one of the disinfectants subtracting the microorganisms initials of the surviving microorganisms and was multiplied by 100.

Statistical analysis

The germicidal efficiency was calculated and to determine what is the best disinfectant between each of the different applications of study, we used a factorial design 32 was used analysis of variance (ANOVA) for each of the three stages of contact was worked with the statistical package Minitab 16.

To calculate the germicidal efficiency the following formula was used:

$$\text{Efficiency (\%)} = (\text{No-Nt})/\text{No} \times 100$$

Where:

No=Number of microorganisms initials

Nt=Number of microorganisms survivor in the time t

Results and Discussion

The germicide efficiency of inert contact surfaces

Table 2 shows the germicidal efficiency of the disinfectants in contact with the product. It can be appreciated that there is no significant difference between times of contact and the disinfectants.

Times of contact (minutes)	Germicidal efficiency %		
	Disinfectants		
	A	B	C
	1%	3%	0,8%
1	99,99	99,99	99,99
	99,99	99,99	99,99
	99,99	99,99	99,99
5	100,00	99,99	99,99
	100,00	99,99	99,99
	100,00	99,99	99,99
10	100,00	99,99	99,99
	100,00	99,99	99,99
	100,00	99,99	99,99

Table 2: Germicidal efficacy of surfaces in contact with the product.

Result showed there was not significant differences in the germicidal efficiency among disinfectants. The disinfectant A (quaternary ammonium) is effective to 5 to 10 minutes of exposure against bacteria of *Escherichia coli* and *Staphylococcus aureus* at a concentration of 1%. Echeverri [15] found for the set times of 5, 10 and 15 minutes a total reduction of populations of *C. albicans*, *S. aureus* and *E. coli* with some disinfectants including quaternary ammonium. Taboada [16] also worked with commercial disinfectants on based quaternary ammonium and bacteria *E. coli* with a time of destruction of 20 minutes. Lopez et al. [12] found a 99.999% reduction against *S. aureus* and *E. coli* in 10 minutes after of applying disinfectants quaternary ammonium base.

Studies results show of the efficiency of quaternary ammonium compounds in suspension against Listeria according to Best [17] and on surfaces according to Krysinski et al. [18], depending on the concentration, pH and temperature of the working solution and the type of diluent water used in the test. Aarnisalo et al. [13] and finally Du et al. [19] study three quaternary ammonium disinfectant and alcohol quaternary ammonium base, where showed a reduction of 1.3 log CFU/g of *Salmonella* populations.

Also Krysinski et al. [18] showed that complete destruction of biofilms of *Listeria monocytogenes* in plastic and stainless steel occurs when the surfaces are first treated with an appropriate detergent, followed by the application of disinfectants.

The quaternary ammonium disinfectant has been reported as effective for bacterias Gram-positive and Gram-negative as corroborated with this study.

The germicide efficiency of living surfaces (hands)

Table 3 shows the calculated values for germicidal efficiency, the results of the analysis of variance indicates that no significant difference to the exposure time. In the comparing between different disinfectants, was observed that disinfectant change significantly influences the response variable. There significant difference between disinfectants, it is suggested to use the disinfectant F, for an exposure time of 2 minutes, and which provides increased germicidal efficacy in living surfaces (hands).

Times of contact (minutes)	Germicidal efficiency %		
	Disinfectants		
	D	E	F
	1,7%	1,7%	1,7%
0,5	48,13	78,40	99,98
	47,33	76,82	99,98
	42,67	76,19	99,98
	22,00	77,5	99,99
1	52,50	79,29	99,99
	40,00	80,63	99,99
	25,00	91,48	100,00
2	42,00	89	100,00
	44,00	92,34	100,00

Table 3: Germicidal efficiency of living surfaces (hands).

The study showed that the best disinfectant is F (quaternary ammonium alcohols) with an exposure time of 2 minutes and a concentration of 1.7%, [20] showed that by inoculating 7.0 UFC/cm^2

populations of *Salmonella* and *E. faecium* obtained a reduction of 1.3 UFC/cm^2 using exposure times of 30 s, 1 min and 5 min with alcohol-based disinfectants quaternary ammonium.

According to Sansebastiano et al. [21] quaternary ammonium compounds are generally used in concentrations ranging from 50 to 500 ppm and contact times ranging from 1 to 30 minutes to assure a good disinfecting effect. Park in 2005 [22] showed that the compounds of alcohol and quaternary ammonia and hydrogen peroxide are effective against *Staphylococcus aureus* ATCC 6538 and *E. coli* ATCC 10536 in the recommended concentrations of use, confirming the present study, in the which is worked with the same bacterias. Zabala et al. [23] indicated that quaternary ammonium compounds are used at 39% in the food industries.

The germicidy efficiency of disinfectants in footbaths

In Table 4 presents the estimated percentages to the footbaths germicidal efficiency. The results of the analysis of variance shows that there is no significant difference to the exposure time.

Times of contact (minutes)	Germicidal efficiency %		
	Disinfectants		
	G	H	I
	0,5%	0,3%	0,5%
1	99,53	99,99	99,95
	99,46	99,99	99,93
	99,43	99,99	99,90
	99,99	100,00	99,95
10	99,99	100,00	99,94
	99,99	100,00	99,95
	99,99	100,00	100,00
15	99,99	100,00	100,00
	99,99	100,00	100,00

Table 4: Germicidal efficiency of footbats.

Studies conducted by Johns [24] on to germicidal efficacy four quaternary ammonium compounds compared with two hypochlorites against *Staphylococcus aureus*, *Bacillus panis*, *Micrococcus candidus*, and *E. coli* showed that against Gram-positive species the quaternary compounds are generally more effective than hypochlorite, against Gram-negative species was reversed, three of four quaternary ammonium were comparable in efficacy.

Payne [25] showed that disinfectants reduced populations of aerobic bacteria, also indicated that the type of disinfectant and exposure time and the presence or absence of organic matter are important considerations when applications include chemical disinfectants on a sanitation program.

Conclusions

For surfaces in contact with the product, no significant difference between disinfectants and between the contact times.

Among the disinfectants used to surfaces that are in contact with the product is recommended quaternary ammonium with a concentration of 1% with an exposure time of 5 minutes.

In the case of living surfaces (hands) the best disinfectant was alcohol including in its composition quaternary ammonium compound at 1.7% with an exposure time of 2 minutes. For footbaths the disinfection treatment corresponded to benzyl-C12-C16-alkyl-dimetityl-ammoniumchloride at 0.3% with an exposure time of 15 minutes shown the best results. Disinfectants containing quaternary ammonium compounds are against bacteria of *Staphylococcus aureus* and *Escherichia coli.*

Acknowledgements

We appreciate the cooperation of Dr. Peter Feng from FDA and ECOLAC Dairy Plant.

References

1. Fuhrman E (2012) Sanitized properly. Processors need to avoid the common traps in sanitation. Food Safety.
2. Entis P (2002) Protecting the environment. Food Microbiology-The laboratory Washington, Food processors Institute, pp. 129-140.
3. McDonnell G, Russell AD (1999) Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 12: 147-179.
4. Puig J (2002) Ingeniería, autocontrol y auditoria de la hygiene en la industria alimentaria. Madrid.
5. Taylor JH, Rogers SJ, Holah JT (1999) A comparison of the bactericidal efficacy of 18 disinfectants used in the food industry against Escherichia coli O157:H7 and Pseudomonas aeruginosa at 10 and 20 degrees C. J Appl Microbiol 87: 718-725.
6. Marriott N (2003) Principios de higiene alimentaria. Zaragoza-España.
7. Troya J (2007) Evaluación de la efectividad de los desinfectantes DIVOSAN FORTE y MH en la desinfección de equipos y áreas de trabajo en una empresa procesadora de helados. Pontificia Universidad Javeriana. Bogotá.
8. Salas DI (2007) Evaluación de metodologías de control higiénico de superficies alimentarias y adopción de la PCR en tiempo real como método de control de patógenos. Tesis Doctoral. Departamento de Ciencia Animal y de Alimentos de la Facultad de Veterinaria de la Universidad Autónoma de Barcelona. Barcelona-España.
9. Wildbrett G (2000) Limpieza y desinfección en la industria alimentaria. Zaragoza.
10. Rojas RC (2007) Evaluación de 4 desinfectantes sobre Listeria monocytógenes aislada de productos cárnicos crudos de una planta procesados en Bogotá. Microbiología industrial.
11. Gibson H, Taylor JH, Hall KE, Holah JT (1999) Effectiveness of cleaning techniques used in the food industry in terms of the removal of bacterial biofilms. J Appl Microbiol 87: 41-48.
12. López L, Romero J, Ureta F (2002) In vitro germicide action of disinfectant products used in the food industry. Arch Latinoam Nutr 52: 74-76.
13. Aarnisalo K, Salo S, Miettinen H, Suihko MJ, Wirtanen G, et al. (2000) Bactericidal efficiencies of commercial disinfectants against *Listeria monocytogenes* on surfaces. Journal of Food Safety 20: 237-250.
14. Food and Drug Administration, USA (1998) Bacteriological Analytical Manual (8thedition.) Revision A, Chapter 3.
15. Echeverri LC, Cifuentes GC, Granados JM, Arias J, Fernández C (2007) Cinética de desinfección para cinco desinfectantes utilizados en industria farmacéutica. Rev Cubana Farm 41.
16. Taboada A, Sanchez E, Cava R, Marin F, Lopez A (2007) Efectividad de desinfectantes de superficies de los equipos en instalaciones de envasado de productos listos para su consumo. Departamento de Tecnología de

Alimentos. Universidad de Murcia. Departamento de Ingeniería de Alimentos. Universidad Politécnica de Cartagena.

17. Best M, Kennedy ME, Coates F (1990) Efficacy of a variety of disinfectants against *Listeria* spp. Appl Environ Microbiol 56: 377-380.
18. Krysinski E, Brown L, Marchisello T (1992) Effect of Cleaners and sanitizers on Listeria monocytogenes attached to product contact surfaces. Journal of food protection: 55: 246-251.
19. Du WX, Danyluk MD, Harris LJ (2010) Efficacy of aqueous and alcohol-based quaternary ammonium sanitizers for reducing *Salmonella* in dusts generated in almond hulling and shelling facilities. J Food Sci 75: M7-13.
20. Kane D (2012) Evaluation of a sanitizing system using isopropyl alcohol quaternary ammonium formula and carbon dioxide for dry-processing environments.
21. Sansebastiano G, Zoni R, Bigliardi L (2007) Cleaning and disinfection procedures in the food industry general aspects and practical applications. Food Safety 1: 253-280.
22. Park HK, Park BK, Shin HW, Park DW, Kim YS, et al. (2005) Evaluation of effectiveness of sanitizers and disinfectans used in domestic food processing plants. Korean Journal of Food Science and Technology 37: 1042-1047.
23. Jiménez ZA, Otazua FM, Maiztegi GP, Serrano IE, Juaristi AA, et al. (2011) Situation of environmental and food area disinfectants registered in Spain under directive 98/8/CE. Rev Esp Salud Publica 85: 175-188.
24. Johns CK (1947) Studies comparing the sanitizing efficiencies of hypochlorites and quaternary ammonium compounds. Can J Res 25: 76-91.
25. Payne J, Kroger E, Watkins S (2005) Evaluation of disinfectant efficacy when applied to the floor of poultry grow-out facilities. J Appl Poult Res 14: 322-329.