pISSN 1229-1153 / eISSN 2465-9223 *J. Food Hyg. Saf.*
Vol. 31, No. 6, pp. 471~475 (2016)
https://doi.org/10.13103/JFHS.2016.31.6.471

Journal of Food Hygiene and Safety
Available online at http://www.foodhygiene.or.kr

Bactericidal Efficacy of a Powder Disinfectant Containing Phosphate Compounds Against *Salmonella* Typhimurium

Eun-Kee Park[1], Chun-Nam Cha[2], Chang-Yeul Yoo[3], Suk Kim, and Hu-Jang Lee*

Institute of Animal Medicine, College of Veterinary Medicine, Gyeongsang National University, Chinju 52828, Korea
[1]Department of Medical Humanities and Social Medicine, College of Medicine, Kosin University, Busan 49267, Korea
[2]Engineering Research Institute, Department of Industrial Systems Engineering, Gyeongsang National University, Chinju 52828, Korea
[3]Department of Computer Information, Gyeongnam Provincial Namhae College, Namhae 52422, Korea

(Received September 9, 2016/Revised September 17, 2016/Accepted September 26, 2016)

ABSTRACT - *Salmonella* Typhimurium (*S.* Typhimurium) is one of the common food pathogens which may cause gastroenteritidis in human and animals. The microorganism also causes the economic loss in animal farming and food industry. In this study, the disinfection efficacy of a powder disinfectant containing phosphate compounds as a main ingredient, was evaluated against *S.* Typhimurium. A bactericidal efficacy test by broth dilution method was used to determine the lowest effective dilution of the disinfectant following exposure to test bacteria for 30 min at 4°C. The disinfectant and test bacteria were diluted with hard water (HW) or organic matter suspension (OM) according to treatment condition. On HW and OM conditions, the bactericidal activity of the disinfectant against *S.* Typhimurium was 4- and 2-fold dilutions, respectively. As the disinfectant composed to phosphate compounds possesses bactericidal efficacy against pathogenic bacteria such as *S.* Typhimurium, the powder disinfectant can be used to control the spread of bacterial diseases.

Key words : Powder disinfectant, Phosphate compounds, *Salmonella* Typhimurium, Disinfectant efficacy

The bacterium *Salmonella* Typhimurium (*S.* Typhimurium) extensively causes self-limiting enteritis, fatal infection in animals, food-borne infection, and typhoid fever in humans[1-3]. The bacterium is a Gram-negative, motile, non-sporing, non-capsulated bacillus that can be contracted through contaminated water, milk, food or fruits and vegetables or via convalescent or chronic carriers[4,5].

Salmonella infections of food animals play an important role in public health and particularly in food safety, as food products of animal origin are considered to be the major source of human *Salmonella* infections[6]. *S.* Typhimurium is one of the most frequently isolated serotypes from pig farms, slaughtered swine, and associated with human foodborne diseases[7,8]. Salmonellosis in livestock animals and human cause enormous economic loss in the world[9]. In the United States and Korea, the total cost for the illness, including medical costs and loss of productivity, has been estimated at $11.4 and 1.01 won billion per annum, respectively[10,11].

In worldwide, antibiotic-resistant *Salmonella* spp. are increasing due to abuse and overuse of antibiotics. Therefore, the effective cleaning and disinfection regimes are essential in the prevention of infections and outbreaks[12,13]. The cleaning and disinfectant regimes depend on the proper use of biocides, and there is the concern that the resulting increased use of biocides in farming, food production, and hospital settings, and the home could contribute to the selection of antibiotic-resistant strains as some mechanisms of biocide resistance also confer antibiotic resistance[14]. Biocides are often composed of a mixture of ingredients that act upon a wide range of cellular mechanisms and targets, which makes it difficult for bacteria to become resistant to biocides[9].

The use of disinfectant is very effective for successful control of diseases from bacteria, fungi and parasites in farm animals[15]. Many disinfectants containing chemical components have been used for decontamination of farmed animal and food borne diseases[16]. However, there is not the efficacy test for the environmentally friendly disinfectant containing phosphate compounds against *S.* Typhimurium. Phosphates are frequently used as additives in order to improve technological and functional qualities of foodstuffs in the production of food[17].

Therefore, this study was carried out to examine bactericidal efficacy of a powder disinfectant against *S.*

*Correspondence to: Hu-Jang Lee, College of Veterinary Medicine, Gyeongsang National University, 501 Jinju dae-ro, Chinju 52828, Korea
Tel: 82-55-772-2352, Fax: 82-55-772-2308
E-mail: hujang@gnu.ac.kr

Typhimurium.

Materials and Methods

Bacteria and culture

S. Typhimurium (G-B-14-21-62) was obtained from the Korean Veterinary Culture Collection (KVCC, Seoul, Korea). The strains were maintained as frozen glycerol stock. The bacteria were cultured in Luria-Bertani (LB) broth containing 1.5% agar and incubated at 37°C.

Disinfectant

The tested disinfectant, Stalosan-F® powder, is composed of phosphate compounds (85%), copper sulfate (2.5%), ferrous sulfate (2.1%), active chlorine (0.25%), and aluminum-silicate (10.15%). The disinfectant was provided by Sung-Won Co., Ltd. (Seoul, Korea). The powder disinfectant was stored in room temperature and prepared for dilution on the day of evaluation. Determination of the antimicrobial efficacy of the disinfectant was based on Animal and Plant Quarantine Agency (Anyang, Korea), Regulation No. 2013-34.

Diluents and treatment condition

Testing was based on bactericidal effects of disinfectant diluents in two treatment conditions (standard hard water (HW) condition and organic matter (OM) condition) and pathogen control (disinfectant negative control) in Table 1. HW, an ingredient of HW treatment condition, was made by adding anhydrous $CaCl_2$ 0.305 g and $MgCl_2·6H_2O$ 0.139 g into one liter distilled water. Organic suspension, an ingredient of OM treatment condition, is a solution of 5% (w/v) yeast extract in HW. The test organism was prepared by titration of the cultural broth into at least 10^8 CFU/mL viable bacteria with the same kind of diluents of treatment condition.

Experimental procedures

For the efficacy test against *S.* Typhimurium, the disinfectant was completely dissolved in the same volume of HW and OM, respectively, and then each solution was diluted 1:1.5, 1:2, 1:2.5, 1:3 and 1:3.5 with HW and OM, correspondingly. To verify the lowest effective dilution of the disinfectant, five serial dilutions of the disinfectant were prepared and placed at 4°C prior to test reaction. 2.5 ml of each disinfectant dilution was mixed with the same amount of test organism followed by contact time of 30 min at 4°C. During this period, the mixture was shaken at 10 min interval. At the end of 30 min contact period, one mL of the mixture was neutralized with 9 mL of Nutrient broth containing 5% inactivated horse serum (BD Korea Co., Ltd., Incheon, Korea) at 37°C. 0.1 mL of the neutralized reaction mixture was subcultured into 10 mL of recovery cultural broth at 37°C for 48 h in incubator. For the test of bacteria control, 2.5 ml of hard water was mixed with the same amount of test organism followed by contact time of 30 min at 4°C, and then all procedure were undertaken in parallel for the disinfection test.

Table 1. Experimental design for the determination of the bactericidal efficacy of the powder disinfectant containing phosphate compounds

Treatment condition[1)]	Contents according to treatment condition[2)]			
	HW	OM	Disinfectant	Bacteria
HW condition	+	-	+	+
OM condition	+	+	+	+
Bacte control	+	-	-	+

[1)] HW: standard hard water, OM: organic matter.
[2)] +: presence, -: absence.

The valid dilution of the disinfectant was determined that the greatest dilution showing no growth in more than four replicates of five tubes per treatment was confirmed. The final dilution time was statistically determined by a median value among three valid dilution of the triplicate test, but each value of which should be within 20% experimental error. In each bacteria control, the number of bacterial growth in the five replicates was counted.

Results

Table 2 shows the final valid dilution of the disinfectant containing phosphate compounds. When the bactericidal effect on HW condition was evaluated, the antibacterial activity of the disinfectant showed on 4-fold dilutions against *S.* Typhimurium. With the investigation of the bactericidal effect of the disinfectant on OM condition, *S.* Typhimurium was inactivated on 2-fold dilutions. As the organic material interferes with efficacy by either inactivating the disinfectant or blocking it from surface contact, the bactericidal activity of the disinfectant on the OM condition was lowered against animal pathogenic bacteria compared with HW condition.

In the bacterial control, the number of *S.* Typhimurium was more than 2.0×10^5 colony forming units (CFU)/mL, which was satisfied with Animal and Plant Quarantine Agency, Regulation No. 2013-34.

Discussion

The powder disinfectant composed to phosphate compounds, copper sulfate, ferrous sulfate, and active chlorine, was a potential antibacterial disinfectant. The antibacterial effect on phosphate works according to the principle of chelation of mainly divalent metal ions (Ca^{2+}, Mg^{2+}), which

Table 2. Final valid dilution of the powder disinfectant containing phosphate compounds

Treatment condition[1]										
HW				OM				BC		
DF	1	2	3	DF	1	2	3	1	2	3
2	○[2]	○	○	2	○	○	○	+[4]	+	+
3	○	○	○	3	×	○	×	+	+	+
4	○	○	○	4	×	×	×	+	+	+
5	×[3]	×	○	5	×	×	×	+	+	+
6	×	×	×	6	×	×	×	+	+	+
7	×	×	×	7	×	×	×	+	+	+
Valid		400		Valid		5.0			+	

[1] HW: standard hard water, OM: organic matter, BC: bacterial control, DF: dilution factor.
[2] No growth in more than four replicates of five tubes per treatment.
[3] Growth in more than two replicates of five tubes per treatment.
[4] All growth in each replicate.

can cause inactive to some essential physiological processes of bacterial growth[18]. The oligodynamic effect was discovered in 1893 as a toxic effect of metal ions such as copper, iron, zinc, silver and gold on bacteria and viruses, even in relatively low concentration[19]. In addition, active chlorine compounds are well-known antimicrobial agents in human medicine and food industry[20].

The antibacterial mechanism of polyphosphates is caused by binding of polyphosphates to the cell wall of early-exponential phase bacterial cells, resulting in bactericidal and bacteriolytic effects[21]. Recent studies indicate that different metals, including copper and iron cause discrete and distinct types of injuries to microbial cells as a result of oxidative stress, protein dysfunction or membrane damage[22,23]. Chlorine is used in water treatment to disinfect drinking water, swimming pools, ornamental ponds and aquaria, sewage and wastewater, and other types of water reservoirs[24]. When hypochlorite solutions containing chlorine are added in small amounts to pool water or industrial water systems, the chlorine atoms hydrolyze from the rest of the molecule forming hypochlorous acid, which acts as a general biocide[25].

In the present study, the antibacterial activity of the powder disinfectant showed on 4- and 2-fold dilutions against *S.* Typhimurium, respectively. In a previous study[26], antibacterial activity was tested against S. Tyhimurium using sanitizers which are based on peracetic acid-hydrogen peroxide, sodium dichloroisocyanurate, and didecyldimethylammonium chloride (DDAC), results revealed that compared with washing parsley and cucumbers with water, treatments with all three sanitizers were not effective, resulting in a maximum reduction of only 0.7 log CFU of *S.* Typhimurium. In another previous study[27], inhibitory effects of the hydrosols of thyme, black cumin, sage, rosemary and bay leaf were investigated against *S.* Typhimurium inoculated to apple and carrots, and thyme hydrosol showed the highest antibacterial effect, resulting in a maximum reduction of 1.76 log CFU of *S.* Typhimurium compared with control. In a study for the bactericidal efficacy of an organochlorine disinfectant, the number of *S.* Typhimurium was reduced from 1.4×10^8 CFU/mL to less than 10^2 CFU/mL after exposure of the disinfectant (HOCl 100 ppm) for 10 min.

With the consideration of dosage, concentration and contact period, the powder disinfectant is more effective than peracetic acid-hydrogen peroxide, sodium dichloroisocyanurate, DDAC and thyme hydrosol. However, the bactericidal activity of the disinfectant is lower than that of an organochlorine disinfectant. As the efficacy of the disinfectant against *S.* Typhimurium was investigated *in vitro*, a controlled field trial is required to determine whether use of the disinfectant will be able to reduce new pathogenic bacteria infection in animal farm and food-industry area.

Conclusions

In animal farm and food industry, salmonellosis was a very important disease because of high mortality for farmed animals, zoonoses and economic loss. In the study of the bactericide efficacy test of the powder disinfectant containing phosphate compounds, the results suggest that the disinfectant has a potential bactericidal activity against *S.* Typhimurium. So, the disinfectant can be used to control the spread of zoonotic bacteria such as *S.* Typhimurium.

Acknowledgement

This work was financially supported by Sung-Won Co., Ltd. (Seoul, Korea).

국문요약

살모넬라증은 가축에 심각한 피해를 유발하는 질병으로, 축산업과 식품산업에 많은 경제적 손실을 초래하고 있다. 본 연구에서는, 복합 인산염을 주성분으로 하는 분말 소독제의 *Salmonella* Typhimuriums에 대한 살균효과 시험을 수행하였다.

배지희석법을 이용한 살균효력시험은 4°C에서 30분 동안 시험 세균을 희석 소독제에 노출시켜 소독제의 가장 효과적인 낮은 희석배수를 결정하는 시험이다. 본 분말 소독제와 시험 세균을 처리조건에 따라 경수와 유기물로 희석하여 반응을 시켰다. 유기물 조건에서, *Salmonella* Typhimurium에 대한 소독제의 살균력은 경수조건에서의 살균력과 비교하여 낮게 나타났는데, 이는 유기물들에 의한 소독제의 살균 유효성분에 대한 저해작용에 따른 것으로 사료된다.

분말 소독제는 *Salmonella* Typhimurium과 같은 병원체에 대해 살균효과를 가지므로, 살모넬라증과 같은 세균성 질병의 확산을 제어하는데 효과적으로 이용될 수 있을 것으로 사료된다.

References

1. Cleaveland, S., Laurenson, M.K. and Taylor. L.H.: Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. *B. Biol. Sci.*, **356**, 991-999 (2001).
2. Kim, G.S., Kim, D.H., Lim, J.J., Lee, J.J., Han, D.Y., Lee, W.M., Jung, W.C., Min, W.G., Won, C.G., Rhee, M.H., Lee, H.J. and Kim, S.: Biological and antibacterial activities of the natural herb *Houttuynia cordata* water extract against the intracellular bacterial pathogen *Salmonella* within the raw 64.7 macrophage. *Biol. Pharm. Bull.*, **31**, 2012-2017 (2009).
3. Kim, D.H., Lim, J.J., Lee, J.J., Jung, W.C., Shin, H.J., Lee, H.J., Kim, G.S. and Kim, S.: Antibacterial and therapeutic effects of *Houttuynia cordata* ethanol extract for murine salmonellosis. *Kor. J. Environ. Agricul.*, **27**, 156-162 (2008).
4. Doughari, J.H.: Antimicrobial activity of *Tamarindus indica* L. *Trop. J. Pharm. Res*., **5**, 597-603 (2006).
5. Doughari, J.H and Okafor, N.B.: Antibacterial activity of *Senna siamae* leaf extracts on *Salmonella typhi*. *Afr. J. Microbiol. Res*., **2**, 42-46 (2008)
6. Ibrahim, M.A., Emeash, H.H., Ghoneim, N.H. and Abdel-Halim, M.A.: Seroepidemiological studies on poultry salmonellosis and its public health importance. *J. World's Poult. Res.,* **3**, 18-23 (2013).
7. Katsuda, K., Kohmoto, M., Kawashima, K. and Tsunemitsu, H.: Frequency of enteropathogen detechtion in sucking and weaned pigs with diarrhea in Japan. *J. Vet. Diagn. Invest.*, **18**, 350-354 (2006).
8. Korsak, N., Jacob, B., Groven, B., Etienne, G., China, B., Ghafir, Y. and Daube, G.: Salmonella contamination of pigs and pork in an integrated pig production system. *J. Food Prot*., **66**, 1126-1133 (2003).
9. Giammanco, G., Pignato, S. and Giammanco, G.M.: Recent trends in salmonellosis epidemiology. *J. Prev. Med. Hyg.,* **40**, 19-24 (1999).
10. Scharff, R.L.: Economic burden from health losses due to foodborne illness in the United States. *J. Food Prot.,* **75**, 123-131 (2012).
11. Whitehead, R.N., Overton, T.W., Kemp, C.L. and Webber, M.A.: Exposure of *Salmonella enteric* serovar Typhimurium to high level biocide challenge can select multidrug resistant mutants in a single step. *PLoS ONE*, **6**, e22833 (2011).
12. Park, E.K., Cho, Y. and Lee, H.J.: Bactericidal efficacy of a disinfectant solution composed to povidine-iodine against *Salmonella typhimurium* and *Brucella ovis*. *J. Fd Hyg. Safety*, **29**, 165-169 (2014).
13. Russell, A.D.: Biocide use and antibiotic resistance: the relevance of laboratory findings to clinical and environmental situations. *Lancet Infect. Dis.*, **3**, 794-803 (2003).
14. Ahmad, K.: Control of animal diseases caused by bacteria: Principles and approaches. *Pak. Vet. J*., **25**, 200-202 (2005).
15. Jeffrey, D.J.: Chemicals used as disinfectants: active ingredients and enhancing additives. *Rev. Sci. Tech. Off. Int. Epiz.,* **14**, 57-74 (1995).
16. Suarez, V.B., Frison, L., De Basilico, M.Z., Riveira, M. and Reinheimer, J.A.: Inhibitory activity of phosphates on molds isolated from foods and food processing plants. *J. Food Prot*., **68**, 2475-2479 (2005).
17. Long, N.H.B.S., Gál, R. and Buňka, F.: Use of phosphates in meat products. *Afr. J. Biotechnol.*, **10**, 19874-19882 (2011).
18. Buňkova, L., Pleva, P., Buňka, F., Valašek, P. and Kračmar, S.: Antibacterial effects of commercially available phosphates on selected microorganisms. *Acta Univ. Agric. et Silvic. Mendel. Brun*., **56**, 19-24 (2008).
19. Kar, A.: Pharmaceutical microbiology. New Age International (P) Ltd., New Delhi, pp. 219 (2008).
20. Gottardi, W. and Nagl, M.: Chlorine covers on living bacteria: the initial step in antimicrobial action of active chlorine compounds. *J. Antimicrob. Chemother*., **55**, 475-482 (2005).
21. Obritsch, J.A., Ryu, D., Lampila, L.E. and Bullerman, L.B.: Antibacterial effects of long-chain polyphosphates on selected spoilage and pathogenic bacteria. *J. Food Prot*., **71**, 1401-1405 (2008).
22. Varkey, A.J.: Antibacterial properties of some metals and alloys in combating coliforms in contaminated water. *Sci. Res. Essays*, **5**, 3834-3839 (2010).
23. Lemire, J.A., Harrison, J.J. and Turner, R.J.: Antimicrobial activity of metals: mechanisms, molecular targets and applications. *Nat. Rev. Microbiol*., **11**, 371-384 (2013).
24. Jolli, J. and Jacob, C: Sustainability management for reducing the health hazards in the indoor swimming pools of UAE. *ASS*, **11**, 124-135 (2015).
25. Mahfouz, A.B., Atilhan, S., Batchelor, B., Linke, P., Abel-Wahab, A. and El-Halwagi, M.: Optimal scheduling of biocide dosing for seawater-cooled power and desalination plants.

Clean Technol. Envir., **13**, 783-796 (2011).
26. Shirron, N., Kisluk, G., Zelikovich, Y., Eivin, I., Shimoni, E. and Yaron, S.: A comparative study assaying commonly used sanitizers for antimicrobial activity against indicator bacteria and a *Salmonella* Typhimurium strain on fresh produce. *J. Food Prot.*, **72**, 2413-2417 (2009).
27. Tornuk, F., Cankurt, H., Ozturk, I., Sagdic, O., Bayram, O. and Yetim, H.: Efficacy of various plant hydrosols as natural food sanitizers in reducing *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on fresh cut carrots and apples. *Int. J. Food Microbiol*., **148**, 30-35 (2011).
28. Choi, T.Y.: Biocidal effect of a sanitizer/disinfectant, Food-safe®, against bacteria, yeast, and mycobacteria. *Korean J. Clin. Microbiol.*, **11**, 117-122 (2008).