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Antimicrobial activity of products based on potassium monopersulfate on bacteria associated with avian infections

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Abstract – The objective of this work was to evaluate the antimicrobial activity of four new potassium monopersulfate-based products on bacterial biofilms associated with avian infections, in order to disinfect drinking fountains in poultry farms. Initially, tests were performed in planktonic bacterial cells, to verify the antimicrobial activity and the minimum inhibitory concentration of the products, named PA, PB, PC, and PD. These products were tested on mature biofilms of the avian pathogens *Escherichia coli*, *Salmonella enterica* subsp. *enterica* serovar Enteritidis, and *Listeria monocytogenes*, grown on specimens preserved in acrylic blocks, by counting colony-forming units, scanning electron microscopy, and confocal fluorescence microscopy. All products were effective against the evaluated bacterial species. The PA and PB products inhibited the bacterial growth at ≤ 0.13% concentrations, and PC and PD showed the same effect at ≤ 0.25% concentrations. Furthermore, the PA product was able to eliminate mature biofilms of *S. enterica* subsp. *enterica* serovar Enteritidis and *L. monocytogenes*. The evaluated monopersulfate-based products, notably PA, are effective against bacterial biofilms associated with avian infections and show potential as sanitizers and disinfectants for drinking fountains in poultry farms.

Index terms: antimicrobial activity, avian pathogen, biofilm, drinking fountain, poultry farm.

Atividade antimicrobiana de produtos à base de monopersulfato de potássio em bactérias associadas a infecções aviárias

Resumo – O objetivo deste trabalho foi avaliar a atividade antimicrobiana de quatro novos produtos à base de monopersulfato de potássio em biofilmes bacterianos associados a infecções aviárias, para a desinfecção de bebedouros em granjas avícolas. Inicialmente, realizaram-se testes em células bacterianas planctônicas, para verificar a atividade antimicrobiana e a concentração inibitória mínima dos produtos, denominados PA, PB, PC e PD. Esses produtos foram testados em biofilmes maduros dos patógenos aviários *Escherichia coli*, *Salmonella enterica* subsp. *enterica* serovar Enteritidis e *Listeria monocytogenes*, cultivados em espécimes preservados em blocos de acrílico, por meio da contagem de unidades formadoras de colônias, por microscopia eletrônica de varredura e por microscopia de fluorescência confocal. Todos os produtos foram eficazes contra as espécies bacterianas avaliadas. Os produtos PA e PB inibiram o crescimento bacteriano em concentrações ≤ 0,13%, e PC e PD apresentaram o mesmo efeito em concentrações ≤ 0,25%. Além disso, o produto PA foi capaz de eliminar os biofilmes maduros de *S. enterica*

subsp. *enterica* serovar Enteritidis e *L. monocytogenes*. Os produtos à base de monopersulfato de potássio avaliados, notadamente o PA, são eficazes contra biofilmes bacterianos associados a infecções aviárias e apresentam potencial como sanitizantes e desinfetantes em bebedouros de granjas avícolas.

Termos para indexação: atividade antimicrobiana, patógenos aviários, biofilme, bebedouro, granja avícola.

Introduction

In 2021, the world market of chicken meat had a of 99,901 thousand tonnes production, of which 14,329 thousand tonnes were produced in the Brazilian market (ABPA, 2022). In 2022, Brazil ranked as one of the largest producers of chicken meat, for exporting 4,822 thousand tonnes (ABPA, 2023). In poultry farms, chickens are placed in poultry litter where they sleep, eat, drink water, and defecate, creating a favorable environment for contamination and infections in these birds (Korzeniowski et al., 2022). Most of such infections are caused by bacteria that form biofilms on the floor bedding (traditionally composed of straw, hay, or wood shavings) and in drinking fountains.

Bacteria *Escherichia coli*, *Salmonella enterica* subsp. *enterica* serovar Enteritidis and *Listeria monocytogenes* cause disease in birds and are also related to disease in humans. Certain strains of *E. coli* have a genetic background that characterizes the avian pathogenic *Escherichia coli* (APEC), and these strains are responsible for causing extraintestinal diseases. The antimicrobial resistance of these bacteria has received a significant attention in Brazil, due to the loss of production in poultry farms and the risk of exposure for consumers (Paiva et al., 2016; Barbieri et al., 2021). *Salmonella* spp. are compulsorily notifiable pathogens, since contamination with this species is a critical problem in poultry farms, as they pose a health risk to both animals and consumers (Evran et al., 2021). *Listeria monocytogenes* is an emerging pathogen of food origin; the contamination by these zoonotic bacteria is permeated by contaminated poultry products, and not by direct infections of infected chickens (Sioutas et al., 2023). The contamination with these bacterial pathogens in birds and, consequently, in humans, is a serious threat to public health and poultry production. Therefore, it is essential to eliminate these agents in poultry farms.

The term biofilm is used to describe bacterial populations enclosed in a matrix, adherent to each other and/or to surfaces, and it may be formed by one or several species, reflecting complex communities of microcolonies separated by water-filled channels (Jay, 2005). Environments with greater water availability are conducive to greater biofilm formation, including bird drinkers in poultry farms (Azeredo & Oliveira, 2000; Tran & Webster, 2013).

Bacterial biofilms are among the main concerns in commercial poultry, as they can be sources of infections in birds and, consequently, in humans, and they can generate also economic losses to producers due to sanitary embargoes (Stoodley et al., 2002). It is important to carry out tests for mature biofilms to simulate what happens in the water troughs of poultry farms. It is common for bacterial biofilms to form inside the polyvinyl chloride (PVC) tubes that distribute water to the birds, which can cause infections throughout the farm (Korzeniowski et al., 2022).

Potassium peroxymonosulfate ($2KHSO_5.KHSO_4.K_2SO_4$), or also potassium monopersulfate, is a product widely used in a variety of industrial applications as an oxidizing agent. Monopersulfate alone is not an effective disinfectant like active chlorine compounds; however, when it is activated catalytically, thermally, or photolytically, the radicals formed are powerful oxidants with potent disinfecting action (Anipsitakis et al., 2008).

It is essential to remove these pathogens from the environment of the poultry farm, especially from the drinking fountains, so that there are no economic losses and risks to human health.

The objective of this work was to evaluate the antimicrobial activity of four new potassium monopersulfate-based products, against bacterial biofilms associated with avian infections, in order to disinfect drinking fountains in poultry farms.

Materials and Methods

The analyses were carried out at the Laboratory of Bacteriology and Molecular Biology, of the Research Group on Environmental Microbiology, at the Universidade de Ribeirão Preto (Unaerp), and in the Cell Biology Laboratory of the Universidade de São Paulo (USP).

The products based on potassium peroxymonosulfate were supplied by the Centro de Pesquisa, Desenvolvimento e Inovação Hidrodomi do Brasil, Ribeirão Preto, SP, Brazil. The evaluated products were: PA, which is a mixture of sodium dichloroisocyanurate and potassium monopersulfate; PB, containing potassium monopersulfate, citric acid, sodium hexametaphosphate, and sodium lauryl sulfate; PC, composed of potassium monopersulfate, citric acid, and sodium dichloroisocyanurate. In addition, a product composed of sodium percarbonate, citric acid, sodium lauryl sulfate, and tetraacetylethylenediamine (TAED) – named PD – was used for comparison purposes for antimicrobial activity, since it is the only one commercially available among the evaluated products.

All products were prepared at a concentration of 500 ppm (0.05% m/v), aiming to standardize the concentrations for comparative purposes in the different tests carried out. Sodium dichloroisocyanurate (DC) is the compound present in PA and PC. In this study, it was prepared with no association, to be used as a control and to evaluate the action of the compound itself. Its solution was prepared at 8 ppm concentration, to reach the concentration of chlorinated water, which contains 5 ppm of active chlorine (Pfuntner, 2011).

The antimicrobial potential of the products was evaluated against three avian pathogenic bacterial strains: APEC, *S. enterica* subsp. *enterica* serovar Enteritidis, and *L. monocytogenes*. The strains were cryopreserved at -80°C, in cryotubes containing BHI (brain heart infusion) broth culture medium (Sigma-Aldrich, San Luis, MO, USA) with 15% glycerol.

The microdilution method employing 96-well plates was used to evaluate the minimum inhibitory concentration (MIC), according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2020). The protocol was performed with adaptations, by using Resazurin to reveal the bacterial growth, as described by Pitondo-Silva et al. (2016). Resazurin (7-Hydroxy-3H-phenoxazin-3-one-10-oxide) dye oxidized form is originally blue, indicating the absence of bacterial growth and, after a redox reaction, it passes to a pink or purple color, in its reduced form, indicating bacterial growth.

A bacterial inoculum suspension was prepared in sterilized saline solution from recent bacterial cultures, aiming to obtain turbidity corresponding to the 0.5 McFarland scale (1.5×10^8 CFU mL^{-1}). From this initial suspension, 500 µL was pipetted and transferred to a test tube containing 4.5 mL of sterilized saline solution (1.5×10^7 CFU mL^{-1}).

In the 96-well round-bottom plate, the first column served as a negative control, and 100 µL of Müeller-Hinton broth (Oxoid, UK) was added to the wells of this column to verify the medium sterility. The twelfth column served as a positive control, and 90 µL of the same culture medium plus 10 µL of the inoculum were added to that column wells, for the evaluation of the bacterial growth viability. From the second to the eleventh column, two-fold serial dilutions were performed with the product solutions. The initial concentration was 2% (m/v), covering a concentration range from 2% to 0.004% (m/v). Finally, 10 µL of the bacterial inoculum was added to all tested solutions reaching 100 µL final volume.

After the incubation of 18 hours at 37°C, two qualitative readings were visually performed: one without chromogenic indicators, to observe only the turbidity level of the culture medium, and the other by applying 30 µL of Resazurin dye, after 3 hours of incubation at 37°C.

All tests were performed in triplicate, and the MIC was determined as the concentration equivalent to the well with the lowest concentration of the product that did not allow bacterial growth.

For the determination of the minimum bactericidal concentration (MBC), the wells that did not show visible bacterial growth were selected. A volume of 10 µL of each one was transferred to Petri dishes containing Müller Hinton Agar (Oxoid, UK). The plates were incubated for 24 hours at 37°C and, afterward, a visual evaluation was performed to determine the MBC by verifying the presence or absence of bacterial colonies. The MBC was considered the lowest concentration showing no bacterial growth.

For the bacterial biofilm tests, polyethylene specimens were used that withstand the sterilization process without deforming, instead of polyvinyl chloride (PVC). Biofilms were formed on 1 cm^2 acrylic Poly (methyl methacrylate) specimens previously sterilized. The strains APEC, *S. enterica* subsp. *enterica* serovar Enteritidis, and *L. monocytogenes* were previously reactivated in BHI broth, incubated for 24 hours at 37°C and, subsequently, inoculated in plates

with BHI agar under the same conditions. Afterward, 1 mL of inoculum in BHI broth corresponding to 1 McFarland scale (3×10^8 CFU mL^{-1}) was transferred to 5 mL sterilized tubes containing the acrylic-preserved specimens. The tubes were incubated for 21 consecutive days at 37°C, with daily inoculum changing to obtain dense and mature biofilms.

The biofilm formation on the surface of the specimens was performed in octuplicate, to allow of the statistical analysis of the results, considering each product action against the different bacterial species tested. Specimens to which no bacterium was added were considered as negative controls.

The evaluation of cell viability after microbial adhesion on the surface of the specimens was performed by CFU quantification. After 21 days of incubation, the specimens containing the adhered biofilms were washed with 2 mL of phosphate-buffered saline (PBS), to remove nonadhered planktonic cells. PBS solution was composed of sodium chloride (NaCl), sodium phosphate dibasic (Na_2HPO_4), and sodium phosphate monobasic monohydrate ($NaH_2PO_4.H_2O$), and its pH was adjusted to 7.2.

Initially, the products were tested at 5%, 1%, and 0.1% concentrations. However, all these concentrations completely eliminated bacterial biofilms of all species. The lowest concentration that allowed of the visualization of biofilms and counting of CFUs was 0.05% m/v (500 ppm) for all products. Although the total elimination of biofilms is desirable, it was important to establish the lowest concentration that would allow of the counting of CFUs, to make it feasible comparative analyses among the tested products. Therefore, the concentration of 500 ppm was standardized for biofilm testing. The biofilms were exposed to the products' action by adding 2 mL of PA, PB, PC, and PD, at 500 ppm concentration, and 2 mL of DC at 8 ppm concentration. The solutions containing the products were kept in contact with the specimens for 5 min. For the positive control, 2 mL of PBS was added to the specimen, following the protocol performed with the products. For the microscopic analyses, an additional specimen was prepared for each product tested, including controls, from which biofilms were not collected.

After 5 min of contact with the specimens, the products were removed and the specimens were placed in 5 mL sterile tubes, containing 2 mL of PBS for biofilm collection. To detach the biofilms for further quantification, the tubes containing the specimens were shaken in a vortex-type mechanical tube shaker (Gehaka, São Paulo, Brazil), at maximum speed for 30 s. Afterward, the tubes were placed in an ultrasonic vat (Kondortech, São Carlos, SP, Brazil) for 480 s, to ensure the detachment of biofilms by ultrasonic vibration. In the next step, the collected aliquots of the detached biofilms were subjected to six serial dilutions, to determine the number of CFU mL^{-1} of each bacterial strain. After the serial dilution, 100 µL of each aliquot were inoculated by the spreading method on BHI agar plates, and incubated for 24 hours at 37°C.

After the incubation period, the images of the plates were documented, and the CFUs were counted using the ImageJ program (Schneider et al., 2012). Plates with visible colonies were selected and counted to establish the values (CFU mL^{-1}), which were converted to a logarithmic scale for better statistical analyses of the results.

The morphological analysis of the biofilms adhered to the acrylic-preserved specimens was performed by scanning electron microscopy (SEM). After the treatment with the products, the selected specimens were fixed with 2.5% glutaraldehyde, for 24 hours, at 4°C. Posteriorly, they were washed with PBS to remove the glutaraldehyde solution excess and planktonic cells deposited on the surface of the specimens. The samples were sequentially dehydrated in ethanol solutions at ascending concentrations (30%, 50%, 70%, 80%, 85%, and 100%) for 5 min each. After dehydration, the samples were placed in a desiccator for 72 hours (Huang et al., 2017; Li et al., 2018).

To evaluate the bacterial adhesion, the acrylic-preserved specimens from each experimental group were placed on an aluminum plate, fixed with double-sided carbon adhesive tape, and metalized with gold-palladium alloy (Balt-Tec SCD-050, Florida, USA) for 120 s. After metallization, the samples were analyzed using SEM (JSM-6610LV Scanning Electron Microscope, JEOL, Tokyo, Japan). The surfaces were observed in a secondary electron regime at 10-15 kV voltage with 500X, 2,000X, and 5,000X magnification to generate the photomicrographs. Each sample was observed at five different equidistant points.

Confocal fluorescence microscopy (Leica SP5 Confocal Microscope, Wetzlar, Germany) analysis was performed to verify the presence of live and dead

bacteria adhered to the specimens, after the action of the products and the ability of the products to remove biofilms. After treatment with the products, the specimens were stained with the bacterial viability kit Live/Dead BacLight Bacterial Viability and Counting Kit (Molecular Probes, Eugene, USA) for 15 min, following the protocol described by Joshi et al. (2010). The kit features fluorescence markers that can detect live and dead bacteria. Those markers are SYTO 9 reagent, which stains viable cells in green by penetrating cells with unaltered membranes, and propidium iodide which stains dead cells in red by penetrating cells with harmed membranes.

The results of statistical analyses were performed to compare the action of the products against the tested bacterial species. Firstly, the Kolmogorov-Smirnov's test was performed to verify if the samples had a normal distribution. Afterward, the Levene's test was applied to verify if the variants were homogenous. After confirming the normality and homogeneity of the data, the single-factor analysis of variance test (ANOVA) was applied, comparing whether groups showed statistically significant differences. In addition to the ANOVA, the Tukey-Kramer's post-test was performed to compare all groups, showing which ones had statistical differences. The analyses were performed using the software Graph Pad Prism, version 5, and Microsoft Excel 2016.

Results and Discussion

The samples showed both normal distribution and homogeneity of the values. All groups exhibited significant differences by the obtained p-values, at 5% probability. Thus, by the Tukey-Kramer's post-test, the significant difference between treatments could be noticed, with p-values of 10^{-7} or less, except for *S. enterica* subsp. *enterica* serovar Enteritidis in PC×DC, whose p-value reached 0.032. There was no significant difference for APEC in PD×PC, *S. enterica* subsp. *enterica* serovar Enteritidis in PB×PC and PD×DC, and *L. monocytogenes* in PD×DC (Table 1).

Among the evaluated products, DC (control) was the most effective, leading to bacterial inhibition at concentrations lower than 0.03% (Table 2). Zabot et al. (2018) reported an effective inhibitory activity of sodium dichloroisocyanurate for bacterial species, especially *S. enterica* subsp. serovar Enteritidis at 60 ppm concentration. Among the tested products, PA attained the best results in the MIC test against APEC and *S. enterica* subsp. *enterica* serovar Enteritidis, at 0.03% concentration, and against *L. monocytogenes* at 0.13% m/v. These results can be explained, since PA is a mixture of sodium dichloroisocyanurate with potassium monopersulfate, and sodium dichloroisocyanurate has a stable chemical structure that slowly reacts with organic matter present in water.

Table 1. Tukey-Kramer's post-test for the comparison among effects of potassium monopersulfate-based products on three bacterial species associated with avian infections.

Product[(1)]		p-value		
Group 1	Group 2	Avian pathogenic *Escherichia coli* (APEC)	*Salmonella enterica* subsp. *enterica* serovar Enteritidis	*Listeria monocytogenes*
PB	PA	*	*	*
PB	PD	*	*	*
PB	PC	*	0.270	*
PB	DC	*	*	*
PA	PD	*	*	*
PA	PC	*	*	*
PA	DC	*	*	*
PD	PC	0.090	*	*
PD	DC	*	0.751	0.708
PC	DC	*	**	*

[(1)]PA, sodium dichloroisocyanurate + potassium monopersulfate; PB, potassium monopersulfate, citric acid, sodium hexametaphosphate, sodium lauryl sulfate; PC, potassium monopersulfate, citric acid, sodium dichloroisocyanurate; PD, sodium percarbonate, citric acid, sodium lauryl sulfate and tetraacetylethylenediamine; DC, sodium dichloroisocyanurate. * and **Significant at 0.1% and 5% probability, respectively.

The mentioned reaction leads to a slower release of hypochlorous acid, which penetrates the bacterial cell destroying it, therefore increasing the effectiveness of the disinfection process. This compound does not change the pH of its solution significantly, it presents very low contents of insoluble solids and, above all, it is highly water-soluble which makes its action easier (Zabot et al., 2018). Potassium monopersulfate, in turn, is a salt that has broad-spectrum of antibacterial and antiviral properties; it is active even in the presence of organic matter and does not cause corrosion in metals, and its oxidizing properties compromise the main physical-chemical processes in the microorganisms (Silva et al., 2021).

The antimicrobial activity of sodium dichloroisocyanurate associated with potassium monopersulfate was previously described by Almeida (2020), who showed that its MIC against *Enterococcus faecalis* was 75% lower than sodium dichloroisocyanurate, and 50% lower than potassium monopersulfate alone. This is indicative of the synergism between the salts when they are associated, enhancing their antimicrobial activity in comparison with their respective individual effects.

It should be noticed that PB showed better results than PC, for APEC, *S. enterica* subsp. *enterica* serovar Enteritidis, and *L. monocytogenes*, although both products are based on monopersulfate (Table 2). The difference between them is related to their chemical composition. PB contains citric acid, sodium hexametaphosphate, and sodium lauryl sulfate, while PC has potassium monopersulfate, citric acid, and sodium dichloroisocyanurate in its formulation. Citric acid is a water-soluble compound, and the acid itself and its formed salts have an antimicrobial activity that controls pathogens in fresh and processed chicken meat. Nevertheless, its use is potentially limited by the requirement to keep the pH low for optimal antimicrobial activity (Zabot et al., 2018). Sodium hexametaphosphate is a cyclophosphate with bactericidal and bacteriostatic properties (Lorencová et al., 2012). Finally, sodium lauryl sulfate is a surfactant with antimicrobial activity, which can inhibit bacterial enzymes associated with fluoride release (Gaetti-Jardim Júnior et al., 1998).

Although PD – the control product – showed some components in common with the other products, without monopersulfate it was less effective in the antimicrobial activity. This can be easily shown by its MICs, whose values hit the concentration of 0.25% m/v for APEC and *L. monocytogenes* (Table 2). These values are higher than he values reached by the PA and PB. Sodium percarbonate, present in PD, when dissolved in an aqueous medium, forms carbonate ions (CO_3^{2-}) and hydrogen peroxide (H_2O_2). The release of these compounds conducts a mechanical cleaning and, at the same time, produces an antimicrobial activity (Sesma & Morimoto, 2011/2012; Fu et al., 2015). However, TAED is an oxygen-release potentiator that acts as a catalyst in the release of active oxygen, when combined with oxidizing compounds such as sodium percarbonate, which has activity against biofilms and contaminants in water (Pawar et al., 2016).

Considering the values of MBC, it can be stated that the tested products have similar efficiencies, with concentrations ranging from 0.25% m/v to 0.5% m/v (Table 2). All tested products (PA, PB, PC, and PD), are innovative and they were not previously reported in the literature.

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) results for potassium monopersulfate-based products against bacterial species associated with avian infections.

Bacterial species	Product[1]									
	PA (%)		PB (%)		PC (%)		PD (%)		DC (%)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli APEC[2]	0.03	0.50	0.06	0.50	0.13	0.50	0.25	0.25	0.02	0.50
Listeria monocytogenes	0.13	0.25	0.13	0.25	0.13	0.25	0.025	0.25	0.03	0.50
Salmonella enterica subsp. *enterica* serovar Enteritidis	0.03	0.50	0.06	0.50	0.25	0.50	0.13	0.25	0.02	0.25

[1]PA, sodium dichloroisocyanurate + potassium monopersulfate; PB, potassium monopersulfate, citric acid, sodium hexametaphosphate, sodium lauryl sulfate; PC, potassium monopersulfate, citric acid, sodium dichloroisocyanurate; PD, sodium percarbonate, citric acid, sodium lauryl sulfate and tetraacetylethylenediamine; DC, sodium dichloroisocyanurate. [2]Avian pathogenic *Escherichia coli*.

At 0.005% concentration, the specimens exposed to the products for 5 min showed plaques with countable CFU for all bacterial species. Nonetheless, PA was the only product that did not show colony counts for *S. enterica* subsp. *enterica s*erovar Enteritidis, and *L. monocytogenes* (0 CFU mL^{-1}), but only for APEC (2.52 CFU mL^{-1}). As expected, the plates of the positive controls, which were exposed to PBS, had uncountable CFUs (Table 3).

Biofilms show more tolerance to antimicrobial agents than planktonic cells. In this sense, it has been postulated that the antibiotic concentrations required to inhibit or kill bacteria in biofilms may be from 100-fold to 1000-fold greater than those required to inhibit or kill planktonically grown strains (Aires et al., 2017, Uruén et al., 2021). All bacterial species evaluated were able to form biofilms on the specimens, according to the SEM analyses. Therefore, it was possible to evaluate the effect of the products on the biofilm structures (Figure 1). The product PA was the most effective to disrupt the structure of biofilms, considering all three bacterial species, and the control DC.

The presence of dead bacteria (red) was observed over the live ones (green) with the live/dead assay, by using the confocal fluorescence microscopy (Figure 2). Treatments with the tested products had a lower incidence of live bacteria than the positive controls and DC. The confocal fluorescence microscopy technique was useful to show whether the biofilms adhered to the specimens were viable and, consequently, capable of causing infection in chickens or not. It is not possible to get these data from SEM, as the biofilm may have remained adhered to the specimen, even suffering the product effect but with no removal. By the use of live/dead kit, it was possible to show that all products were able to kill bacterial cells adhered to the specimens (Figure 2). However, PA had greater effectiveness in disrupting biofilms than the other products, including the positive control and DC. Comparatively, there are more dead than living cells in specimens subjected to PA than the other products.

For APEC, by the post-test, the product with the best result was PA, with 2.52 CFU mL^{-1} average, which was different from all other treatments. PC and PD exhibited a mean of 5.36 and 5.14 CFU mL^{-1}, respectively; however, without difference between them. The evaluated products had better antimicrobial activity than the control DC (6.02 CFU mL^{-1}), except for PB that was less efficient than the other products, with 7.57 CFU mL^{-1} average.

For *S. enterica* subsp. *enterica* serovar Enteritidis, PA was the most effective treatment, as it showed no bacterial growth on the plates and eliminated the bacterial biofilm. PB and PC had averages of 5.53 and 5.68 CFU mL^{-1}, respectively. PD showed 6.19 CFU mL^{-1} average, while DC reached 6.04 CFU mL^{-1} for *S. enterica* subsp. *enterica* serovar Enteritidis. The products PA, PB, and PC had a more efficient antimicrobial activity than the control DC.

For *L. monocytogenes*, PA treatment had no CFU count, followed by PB with an average of 3.93 CFU mL^{-1}, and PC, with 4.56 CFU mL^{-1} (Table 3). The CFU average counts for the treatment PD and control were 5.12 and 5.08 CFU mL^{-1}, respectively. Hence, PA, PB, and PC showed more efficient antimicrobial actions for biofilm disruption than the control DC. It is also noteworthy that the PA had the lowest averages for CFU counts for all studied bacteria, which indicates its greater effectiveness.

Table 3. Counts (log_{10}) of the average colony forming units (CFU) obtained for three bacterial species associated with avian infections, after exposure to potassium monopersulfate-based products.

Bacterial species	Product[1]				
	PA	PB	PC	PD	DC
Escherichia coli APEC[2]	2.52	7.58	5.36	5.14	6.02
Salmonella enterica subsp. *enterica* serovar Enteritidis	0	5.53	5.68	6.16	6.04
Listeria monocytogenes	0	3.93	4.59	5.12	5.08

[1]PA, sodium dichloroisocyanurate + potassium monopersulfate; PB, potassium monopersulfate, citric acid, sodium hexametaphosphate, sodium lauryl sulfate; PC, potassium monopersulfate, citric acid, sodium dichloroisocyanurate; PD, sodium percarbonate, citric acid, sodium lauryl sulfate and tetraacetylethylene diamine; DC, sodium dichloroisocyanurate. [2]APEC, avian pathogenic *Escherichia coli*.

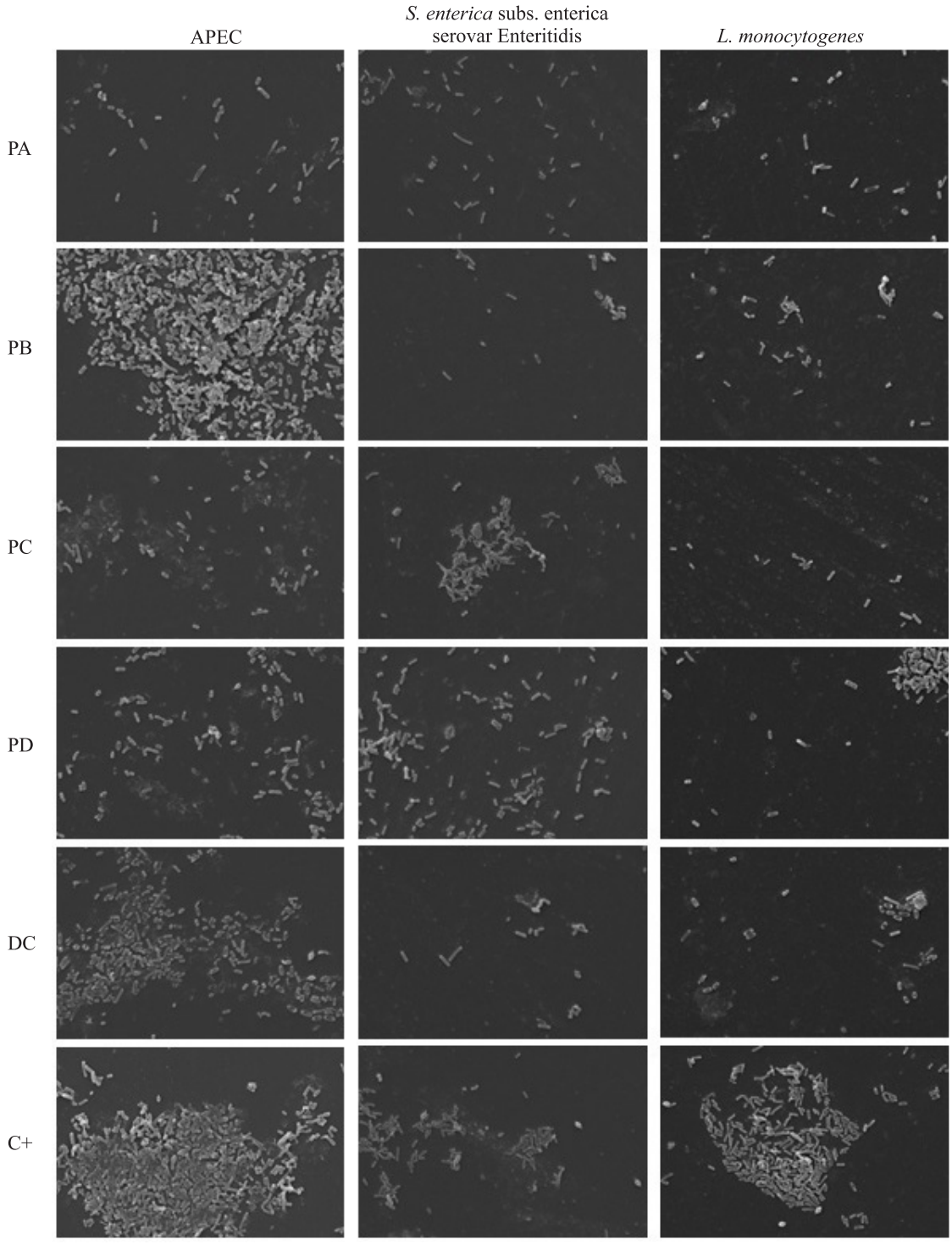

Figure 1. Biofilms adhered to the acrylic-preserved specimens after treatment with the products for three bacterial species (APEC – avian pathogenic *Escherichia coli*, *S. enterica* subsp. *enterica* serovar Enteritidis, and *Listeria monocytogenes*), in images obtained by scanning electron microscopy at 2,000X magnification. Treatments: PA, sodium dichloroisocyanurate + potassium monopersulfate; PB, potassium monopersulfate, citric acid, sodium hexametaphosphate, sodium lauryl sulfate; PC, potassium monopersulfate, citric acid, sodium dichloroisocyanurate; PD, sodium percarbonate, citric acid, sodium lauryl sulfate, and tetraacetylethylenediamine; DC, sodium dichloroisocyanurate; C+ (positive control), phosphate-buffered saline (PBS).

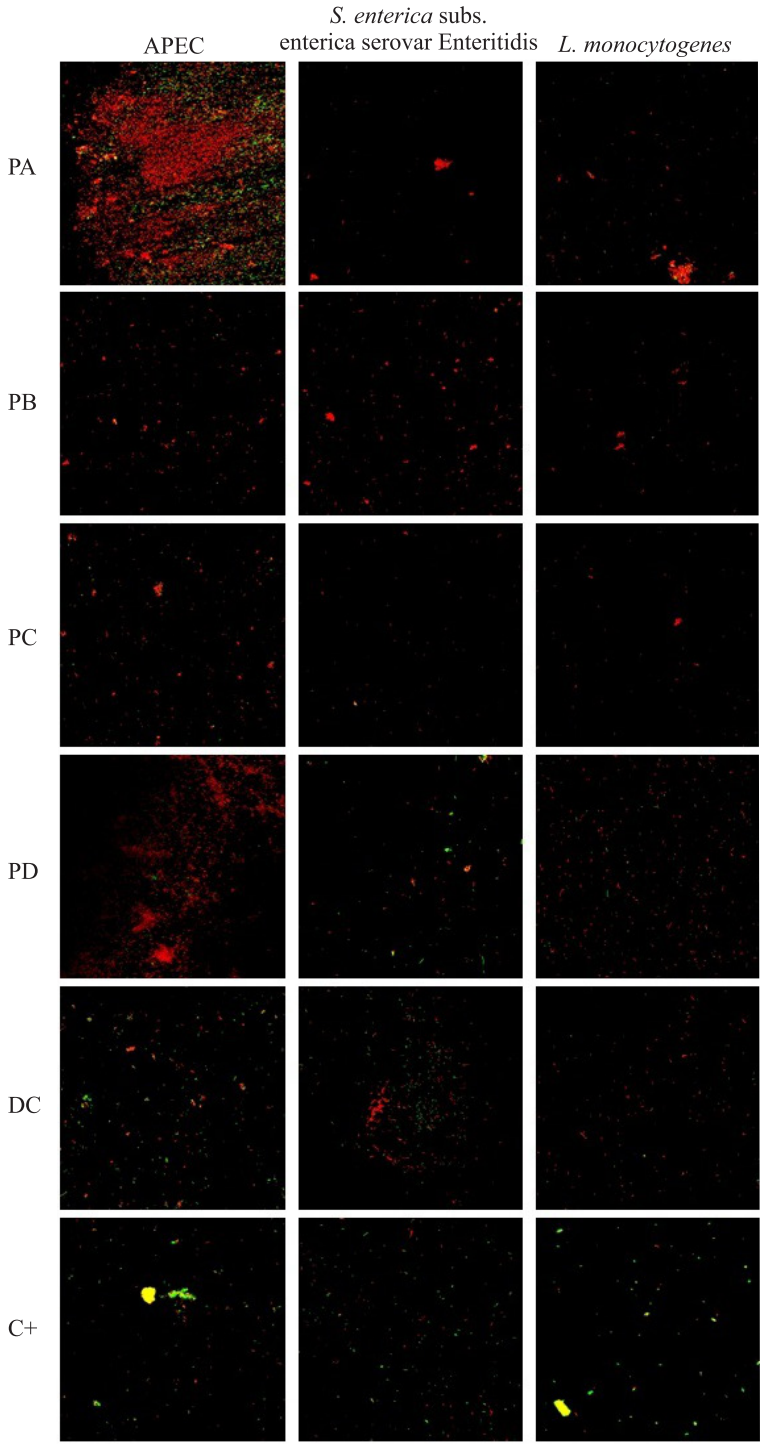

Figure 2. Viable and nonviable cells after treatment with the products for three bacterial strains (APEC – avian pathogenic *Escherichia coli*, *S. enterica* subsp. *enterica* serovar Enteritidis, and *Listeria monocytogenes*), in images obtained by confocal microscopy. Green color: viable (live) cells. Red color: nonviable (dead) cells. Yellow color: overlapping of viable and nonviable cells. Treatments: PA, sodium dichloroisocyanurate + potassium monopersulfate; PB, potassium monopersulfate, citric acid, sodium hexametaphosphate, sodium lauryl sulfate; PC, potassium monopersulfate, citric acid, sodium dichloroisocyanurate; PD, sodium percarbonate, citric acid, sodium lauryl sulfate, and tetraacetylethylenediamine; DC, sodium dichloroisocyanurate; C+ (positive control), phosphate-buffered saline (PBS).

Conclusions

1. The evaluated monopersulfate-based products, notably PA, have antimicrobial effectiveness against bacterial biofilms associated with avian infections.

2. The PA product at low concentrations effectively eliminates mature biofilms of *S. enterica* subsp. *enterica* serovar Enteritidis and *L. monocytogenes* avian pathogens.

3. The PA product has the potential for use as both sanitizer and disinfectant in drinking fountains in poultry farms.

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