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Selected Aspects of the Antibacterial Use of Lactic Acid in Food Processing †

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Abstract: L(+)-lactic acid is authorized as an active substance for biocidal products, with applications in veterinary hygiene, food, and animal feed. Several factors can influence the antimicrobial effect of lactic acid, including the applied concentration of lactic acid, the contact time, and the organic soiling of the surface to which it was applied. The aim of this study was to evaluate the bactericidal activity of 5% (*v*/*v*) and 1% (*v*/*v*) lactic acid solutions on test bacteria: the Gram-negative strain *Salmonella enterica* subsp. *enterica* serovar Typhimurium (ATCC 14028) and the Gram-positive *Staphylococcus aureus* subsp. *aureus* (ATCC 33592), which is a strain that is resistant to gentamicin and methicillin (MRSA). A dilution–neutralisation method was used, based on a quantitative suspension test EN 1276, for the evaluation of the bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas, with the bacterial activity being modified in part of the obligatory test organisms. The obtained results showed no difference in the results of the tests under simulated clean and dirty conditions. Both tested lactic acid concentrations showed a 5 lg reduction in *Salmonella* Typhimurium. For the tested strain, *Staphylococcus aureus* (MRSA), the required reduction of 5 lg was not achieved. These results contribute to a better understanding and rationale for the use of lactic acid for antibacterial purposes.

Keywords: bactericidal activity; lactic acid; *Salmonella* Typhimurium; *Staphylococcus aureus* (MRSA)

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1. Introduction

Organic acids, including lactic acid, which are in GRAS (Generally Recognized As Safe) status, have been investigated because of their bactericidal activity [1]. Their antibacterial effect is a consequence of reducing the pH value of the environment and the cell and increasing the accumulation of anions [2]. Lactic acid has significant applications in many industries, such as the cosmetic, pharmaceutical, chemical, food, and, most recently, medical industries [3]. As permitted food-additive lactic acid complies with food standards [4], lactic acid is well known in food technology as a preservative in naturally fermented products or as a technological additive E (270) without quantity restriction. The world market for lactic acid is estimated at 3.46 billion US dollars in 2022 and is expected to increase by 8.70% during the forecast period 2023 to 2032 [5]. Regardless of the knowledge of the exact mechanism of action on microorganisms, lactic acid, among other organic acids, is recognised as a bio-preservative in naturally fermented products [6]. Numerous applications of lactic acid for meat decontamination have been described [7–10]. Even though its liquid form compared to aerosol is less active, lactic acid is used as a spray for environmental decontamination and as an acidulant for fruit juices and foods with a low

pH value [11]. Although lactic acid is widely used as an antimicrobial agent, the question arises about its effectiveness in outbreaks of a certain microorganism and the possibility of its use to disinfect surfaces contaminated by that pathogen.

The aim of this study was to evaluate the bactericidal activity of 5% (*v*/*v*) and 1% (*v*/*v*) lactic acid solutions on the test bacteria: the Gram-negative *Salmonella enterica* subsp. *enterica* serovar Typhimurium (ATCC 14028) and the Gram-positive *Staphylococcus aureus* subsp. *aureus* (ATCC 33592) (MRSA). The objective was to investigate the bactericidal activity of lactic acid solutions against key bacterial strains, with implications for their use in food safety and hygiene in food production facilities.

2. Material and Methods

2.1. Bacterial Suspensions

The performed quantitative suspension test was based on EN 1276 [12] for bactericidal activity except for obligatory microorganism use. The method was adopted in the segment of used test microorganisms, which are not required by the standard EN 1276 [12], and the other criteria were retained. Bactericidal test suspensions were prepared using third passages of strains grown on tryptone soya agar (TSA, Oxoid). The test suspension of *S. aureus* subsp. *aureus* (ATCC 33592), a strain resistant to gentamicin and methicillin (MRSA), had a density of 0.9 McFarland scale. The test suspension of *Salmonella* Typhimurium (ATCC 14028) had a density of 0.5 McFarland scale. One millilitre of the dilutions 10^{-6} and 10^{-7} of test suspensions was poured-plated with melted tryptone soya agar (HiMedia, Mumbai, India) and incubated for 48 h at 37 ± 1 °C to obtain cell numbers in the suspensions. The conducted tests showed satisfactory results within the requirements of standard EN 1276 [12] for ensuring the quality of testing. The averages of estimated cell numbers in three suspensions were 8.36 ± 0.04 CFUmL^{-1} for *Salmonella* Typhimurium (ATCC 14028) and 8.34 ± 0.03 lg CFUmL^{-1} for *S. aureus* (ATCC 33592). The cell numbers of bacteria in suspensions of both strains satisfied the required interval 8.17 and 8.80 lg CFUmL^{-1} stated in EN 1276 [12].

2.2. Test Product

The test was performed immediately on three products with various lactic acid concentrations. Test products were prepared using L(+) lactic acid, p.a. (CAS No. 79-33-4, concentration 80%, Centroprom, Belgrade, Serbia). For the test, prepared water solutions of the target products with the following final concentrations: product I—6.25% (*v*/*v*) lactic acid and product II—1.25% (*v*/*v*) lactic acid. Product III—0.125% (*v*/*v*) lactic acid was used as a concentration with no expected bactericidal activity.

2.3. Method

The dilution-neutralization method of choice was used. Neutraliser was prepared in the laboratory using the following components: L-histidine (CAS No. 9048-46-8, Merck, Darmstadt, Germany), saponin (CAS No. 8047-15-2, Sigma Aldrich, Burlington, MA, USA), Tween 80(CAS No. 9005-65-6, HiMedia), sodium thiosulphate pentahydrate (CAS 10102-17-7, Merck). The interfering substance was albumin bovine fraction V applied in two concentrations: 0.3 gL^{-1} for simulated clean condition and 3.0 gL^{-1} for simulated dirty condition; albumin bovine (CAS No. 9048-46-8, HiMedia). In brief, a mixture was prepared by adding 1.0 mL of microbial suspension with 1 mL interfering substance solution, after 2 min, 8 mL of product was added. After a contact time of 5 min, 1.0 mL of the mixture was added in another mixture of 8.0 mL of neutraliser and 1.0 mL of water. Neutralisation was performed to quench the antimicrobial activity of the product. After 5 min of neutralisation, 1.0 mL was transferred to a Petri plate in a duplicate and pure plate. The test was performed for each microorganism. The plates were incubated for 48 h at 37 ± 1 °C. Tests were performed three times on freshly prepared Products I, II, and III, and results are presented as average values with standard deviations.

2.4. Method Validation

In parallel, each test was performed with three controls as validation of the methods. Validation suspensions are prepared from a dilution of 10^{-5} of each bacterial suspension, following EN 1276 [12]. Validation A to ensure no biocide activity of used water, validation B to ensure no biocide activity of neutraliser, and validation C to confirm that the biocide product was neutralised. Validation A was performed by adding 8.0 mL of sterile water instead of the product. Validation B was performed by adding 8.0 mL of neutraliser and 1.0 mL of water to the microbial suspension. Validation C was performed by adding 1.0 mL microbial suspension to the already neutralised product. From each validation mixture (A, B or C) 1.0 mL was pour-plated in duplicate with melted tryptone soya agar (HiMedia), and the plates were incubated for 48 h at 37 ± 1 °C. The results of validation suspension, validation A, validation B and validation C were in accordance with the requirement of EN 1276 [12].

2.5. Calculation

For each product concentration and each experimental condition was calculated decimal lg reduction, using the following formula:

$$\lg R = \lg (N/10) - \lg (X \times 10), \quad (1)$$

where R, N and X denote reduction (lg CFUmL^{-1}), the count of strain in suspension (lg CFUmL^{-1}), and the count of viable microorganisms after treatment (lg CFUmL^{-1}), respectively.

3. Results and Discussion

Due to the test design and considering the addition of interfering substances and bacterial suspension, the concentrations of products were diluted to 80% (*v*/*v*) of the initial concentration. So, the solutions of 5% (*v*/*v*), 1% (*v*/*v*), and 0.001% (*v*/*v*) of lactic acid were actively tested. The results of tested lactic acid solutions with a contact time of 5 min are presented in Table 1.

Table 1. Results of antibacterial activity test on *Salmonella* Typhimurium (ATCC 14028) and *S. aureus* (ATCC 33592) in contact time of 5 min.

Bacterium	Concentration of Lactic Acid in the Product (% *v*/*v*)	Tested Concentration of Lactic Acid in the Product (% *v*/*v*)	Condition	Reduction *X_{sr} ± SD (lg CFUmL^{-1})
Salmonella enterica subsp. *enterica* serovar Typhimurium (ATCC 14028)	6.25	5	clean	>5.18 ± 0.04
	6.25	5	dirty	>5.18 ± 0.04
	1.25	1	clean	>5.18 ± 0.04
	1.25	1	dirty	>5.18 ± 0.04
	0.001	0.0008	clean	<3.81 ± 0.04
	0.001	0.0008	dirty	<3.81 ± 0.04
Staphylococcus aureus subsp. *aureus* (ATCC 33592)	6.25	5	clean	<3.82 ± 0.03
	6.25	5	dirty	<3.82 ± 0.03
	1.25	1	clean	<3.82 ± 0.03
	1.25	1	dirty	<3.82 ± 0.03
	0.001	0.0008	clean	<3.82 ± 0.03
	0.001	0.0008	dirty	<3.82 ± 0.03

*X_{sr} ± SD denote average value ± standard deviation.

3.1. S. Typhimurim (ATCC 14028)

The tested target concentration of lactic acid showed that for Gram-negative bacterium *S. enterica* subsp. *enterica* serovar Typhimurium (ATCC 14028) reductions in contact time 5 min were more than 5 lg CFUmL^{-1} and satisfied a requirement of standard EN 1276 [12].

Furthermore, the results show that the use of a lower concentration of lactic acid, i.e., 1% (*v*/*v*), showed the same effects as a higher concentration, i.e., 5% (*v*/*v*), which is a significant contribution to rational use and the protection of the environment. So, the presented results are in favour of the bactericidal use of lactic acid against *Salmonella* Typhimurium. *Salmonella* Typhimurium is one of the leading serovars responsible for human and animal salmonellosis and the expansion of antibiotic resistance [13]. The research of Khan et al. [14] concluded that organic acids, including lactic acid, could be a good alternative to antibiotics. Otherwise, contact with contaminated food in a food-processing environment is a source of cross-contamination and biofilm formation as a potential transmission route for food pathogens. Organic acids, including lactic acid, may play an important antibacterial action [15] with high disinfection potential, depending on the treated bacterial strain [16].

3.2. S. aureus (ATCC 33592)—MRSA

The tested concentration of lactic acid did not show bactericide activity against the Gram-positive bacterium *S. aureus* subsp. *aureus* (ATCC 33592)—MRSA. The reductions for both target lactic acid concentrations were below 5 lg $CFUmL^{-1}$. Methicillin-resistant *S. aureus*—MRSA is known for its methicillin resistance. Neubauer et al. [17] performed a test with no soiling following EN 1040 [18] and reported that 5% lactic acid did not show five logs of *S. aureus*, which is similar to the presented results, with the difference that this test was performed with organic soiling. MRSA, also known as HA-MRSA (health-care-associated MRSA), is recognised as a problem in healthcare facilities and causes a difficult-to-treat infection [19]. Some research indicated that infections of community-associated methicillin-resistant *S. aureus* (CA-MRSA, i.e., MRSA colonisation and infection not associated with healthcare settings) confer a substantial economic burden on third-party payers and society [20]. HA-MRSA, CA-MRSA, and HA-MRSA (livestock-associated MRSA) have been found in food [21] and surfaces accompanied by low hygienic practice [22]. Food industry surfaces could be a reservoir for *S. aureus* to form complex communities with undesirable bacteria in multispecies biofilms [23]. Proper cleaning and disinfection are essential for quality assurance in food industry facilities.

3.3. Influencing Factors

The presented results showed a difference in lg reduction depending on the treated bacterial Gram-negative and Gram-positive strains. Alakomi et al. [24] already reported that in addition to antimicrobial properties due to the lowering of the intracellular pH and synergistic effects with other antimicrobial substances, lactic acid also permeabilised the Gram-negative bacterial outer membrane, which is consistent with the results presented in this paper for a tested Gram-negative strain. On the contrary, Singh et Yemmireddy [25] examined the effect of 0.5% lactic acid on the log reduction in *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* cells that were previously subjected to various physiological stresses and compared with control cell growth and indicated that there were no significant differences in reductions among tested food-borne pathogens ($p > 0.05$). So, the results are comparable only in the case of applying the same concentration of lactic acid and contact time for the specified logarithmic reduction in bacteria as the criterion for bactericidal activity.

Đurđević-Milošević et al. [26] reported bactericidal activity of 5% (*v*/*v*) and 1% (*v*/*v*) lactic acid against *Salmonella* Enteritidis (ATCC 13076) and absence of bactericidal activity against *S. aureus* (ATCC 6538). Compared to the presented results, there is an indication that different strains of the same species can give similar results. Besides, 2–5% lactic acid spray solution application on bovine carcasses approved and admitted in Europe [27] can be insufficient for disinfection in cases of high Salmonella contamination. Used tests of the efficacy of the disinfectants can lead to incomparability of the results and differences in conclusions as well as variations in recommendations for use. The antibacterial properties of the disinfectants are influenced by many factors, including the physiological state of

the bacteria and environmental stress, formed biofilm, organic soiling on the surface, cell number, and diversity of bacteria present on contaminated surfaces. The presented results showed no difference in reductions of tested bacteria depending on applied artificial soiling. Testing in simulated clean and dirty conditions showed similar results.

4. Conclusions

This study focused on the evaluation of the bactericidal activity of lactic acid solutions at a contact time of 5 min, against *Salmonella enterica* subsp. *enterica* serovar Typhimurium (ATCC 14028) and *Staphylococcus aureus* subsp. *aureus* (ATCC 33592) (MRSA) and showed the absence of bactericidal activity against Gram-positive strains. Variation of organic soiling as a simulation of clean and dirty soil did not influence variation in results. Also, a lower tested concentration of lactic acid, i.e., 1% (*v*/*v*), showed antibacterial activity as a direction for the use of lactic acid solution for disinfection of surfaces contaminated with Salmonella. The proper use of a lower concentration of lactic acid with a bactericidal effect contributes to the preservation of the environment from chemical pollution.

Although the design of the conducted experiments cannot explain the mechanism of action of lactic acid on bacteria, it provides results that support the idea that the 1% (*v*/*v*) solution of lactic acid at a contact time of 5 min can be a useful tool in preventive and corrective actions associated with Salmonella outbreaks.

Further studies are currently being conducted to investigate the enhancement of bactericidal activity of lactic acid solution in a shorter time with a lower concentration of lactic acid and against various Gram-negative strains. Also, a combination of lactic acid and some surfactants could give valuable information about the presence or absence of a synergistic bactericidal effect.

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