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Original Article
Özgün Araştırma

Invitro decontamination effect of zinc oxide nanoparticles (ZnO-NPs) on important foodborne pathogens

Tahsin Onur Kevenk[1], Ahmet Koluman[2]

[1] Aksaray University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology. TR 68000 Aksaray, Turkey.*
[2] Pamukkale University, Faculty of Technology, Department of Biomedical Engineering, TR 20160 Denizli, Turkey.

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Abstract: Zinc oxide (ZnO) has been used for many years in the pharmaceutical, cosmetic, paint, textile, and food industries for coating surfaces, absorbing UV rays and due to its antimicrobial properties in nanoscale, it has been identified as an important chemical for decontamination. Zinc can be found in many foods as well and its allowed daily intake for adults has been reported as 8-11 mg. Zinc Oxide Nanoparticles (ZnO-NPs) are generally regarded as safe (GRAS) for it being stable under hard processing conditions. Compared to organic acids, ZnO-NPs have better durability, selectivity, and heat resistance. In the present study, it was aimed to understand the decontamination effect of ZnO-NPs on *S. enteritidis, S. typhimurium, S. aureus, L. monocytogenes,* and *E. coli* O157 to develop novel, safe decontamination agents for the food industry. For this purpose, <50 µ ZnO-NPs were added into Tryptic Soy Broth in 20 mMolar final concentration for the understanding of the antimicrobial effect. After inoculation of the pathogens, a counting procedure was performed using the Tryptic Soy Agar by the pour on plate method at 0, 1st, 2nd, 4th, 6th, 8th, 12th, and 24th hours. As a result, *S. enteritidis* and *S. aureus* 3 Log CFU/mL, *S. typhimurium* and *E. coli* O157 4 Log CFU/mL, *L. monocytogenes* 2 Log CFU/mL decreased in 24 hours.

Keywords: Decontamination, foodborne pathogens, nanoparticles, zinc oxide

Çinko oksit nanopartiküllerin (ZnO-NP) önemli gıda kaynaklı patojenler üzerine in vitro dekontaminasyon etkisi

Özet: Çinko oksit (ZnO) ilaç, kozmetik, boya, tekstil ve gıda endüstrilerinde yüzeyleri kaplamak, UV ışınlarını absorbe etmek için uzun yıllardır kullanılmaktadır ve nano ölçekte antimikrobiyal özellikleri nedeniyle dekontaminasyon için önemli bir kimyasal olarak tanımlanmıştır. Çinko, birçok besinde de bulunabilen bir kimyasal olup yetişkinler için izin verilen günlük alım miktarı 8-11 mg olarak bildirilmiştir. Çinko Oksit Nanopartiküller (ZnO-NP'ler) zorlu gıda işleme koşulları altında stabil kalabilmeleri nedeniyle genellikle güvenli olarak (GRAS) kabul edilmişlerdir. Organik asitlerle karşılaştırıldığında, ZnO-NP'ler daha iyi dayanıklılığa, seçiciliğe ve ısı direncine sahiptir. Bu çalışmada, gıda endüstrisi için yeni, güvenli dekontaminasyon ajanları geliştirmek için ZnO-NP'lerin *S. enteritidis, S. typhimurium, S. aureus, L. monocytogenes* ve *E. coli* O157 üzerindeki dekontaminasyon etkisini anlamak hedeflenmiştir. Bu amaçla, antimikrobiyal etkinin anlaşılması için Tryptic Soy Broth içerisine nihai konsantrasyonu 20 mMolar olacak şekilde <50 µ büyüklüğünde ZnO-NP'ler eklenmiştir. Patojenlerin inokülasyonundan sonra, 0., 1., 2., 4., 6., 8., 12. ve 24. saatlerde Tryptic Soy Agar'da dökme plak yöntemi ile bakteri sayımları yapılmıştır. Sonuç olarak, 24 saatte *S. enteritidis ve S. aureus* 3 Log CFU/mL, *S. typhimurium* ve *E. coli* O157 4 Log CFU/mL, *L. monocytogenes* 2 Log CFU/mL azalmıştır.

Anahtar kelimeler: Çinko oksit, dekontaminasyon, gıda kaynaklı patojenler, nanopartikül

Introduction

Particles which dimensions between 1-100 nanometers (nm) are called nanoparticles. It has been reported that, because of their unique physico-chemical features, high surface area, and volume ratio, the nanoparticles have great antibacterial activity in fluid and solid environments (Bharat et al., 2019; El-Mashad, Pan, 2015). Therefore, depending on the improvement of hygiene understanding in the last few decades these nanoparticles have been used in several areas such as biomedical, education, textile, and food industry (Deshmukh, Patil, Mullani, Delekar, 2019).

There are plenty of different types of nanoparticles according to their basic substance it contains. Some inorganic metals (Al, Cu, Au, Fe, Ag, Ti, Zn) and metal oxides (Al_2O_3, CuO, Cu_2O, TiO_2, ZnO) are examples of these (Rajput et al., 2018). In the year 2010, it was considered that between 260,000-309,000 tons of nanoparticles were produced in the world and it was estimated that this amount of number is going to increase 585,000

Yazışma adresi / Correspondence: *Tahsin Onur Kevenk, Aksaray University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology. Aksaray, Turkey E-mail: tahsinonurkevenk@aksaray.edu.tr*

ORCID IDs of the authors: *[1]0000-0003-2519-8060 • [2]0000-0001-5308-8884*

tons by the year 2019 (Yadav T., 2014). Accordingly, zinc oxide nanoparticles (ZnO-NPs) are known as the third most-produced metal-oxide nanoparticles with 33,400 tons per year (Peng, Tsai, Hsiung, Lin, & Shih, 2017). It has been reported that ZnO-NPs are a bio-safe material. On the other hand, according to some researchers, the information about the toxicity of ZnO-NPs are still limited (Rajput et al., 2018). However, the relationship between the size and shape of particle and toxicity has been demonstrated (Khare et al., 2015).

The bacterial cell wall is defined as a cellular structure consisting of a peptidoglycan layer that increases durability and osmotic resistance. According to its structural features and content, the bacterial cell wall is divided into two subgroups, gram-positive and gram-negative (Hajipour et al., 2012). Several studies showed that gram-negative bacteria such as *E. coli* has been found more sensitive to nanoparticles than gram-positive ones like *S. aureus* and *L. monocytogenes* (Baek YW, An YJ 2011). Although the lethal effects of nanoparticles on bacteria are not fully understood, it is thought that the damage occurs as a result of electrostatic interaction between nanoparticles and the bacterial cell wall (Soenen et al., 2011).

Salmonella enteritidis, *Salmonella typhimurium*, *S. aureus*, *L. monocytogenes*, and *E. coli* O157 have been reported as the major foodborne pathogens that cause food poisoning or intoxications in the USA (Prevention, 2019).

Salmonella enteritidis and *Salmonella typhimurium* are members of the Salmonella genus which are gram-negative bacilli, facultative anaerobe, non-spore forming, motile, catalase positive, and oxidase negative. It is known that the Salmonella genus involves more than 2500 serotypes, but *Salmonella enteritidis* and *Salmonella typhimurium* are the most isolated serotypes in human infections (Dar et al., 2017; Hur, Jawale, Lee, 2012).

S. aureus is gram-positive, non-motile, non-spore forming, catalase, coagulase, thermo nuclease positive, coccus shaped bacteria that can be found in the healthy humans' nasal flora (Wu et al., 2016). *S. aureus* can produce more than twenty enterotoxins which are the reason of staphylococcal intoxication (Balaban, Rasooly, 2000; Hennekinne, De Buyser, Dragacci, 2012).

L. monocytogenes is gram-positive, motile, non-spore forming, ubiquitous, psychrotrophic, and in intracellular microorganism which is the reason of an important food-borne disease called listeriosis (Kevenk, Terzi Gulel, 2016). It has been reported that listeriosis can occur because of consuming various source of foods such as dairy products, meat products or unwashed fruits. Furthermore, it has been reported that listeriosis is a long incubation period disease and may cause many symptoms such as abortions, premature births, meningitis, septicemia. Also, listeriosis has a relatively high (20-30%) mortality rate (Gandhi, Chikindas, 2007).

In this research, it is aimed to understand the in vitro decontamination effect of ZnO-NPs on important foodborne pathogens and to guide the development of new types of disinfectants containing nanoparticles for future use.

Materials and Methods

Bacterial Strains

In the present study, three gram-negative and two gram-positive foodborne pathogen such as *Salmonella enteritidis* ATCC 31194, *Salmonella typhimurium* ATCC 14028, *E. coli* O157 ATCC 43895, *S. aureus* ATCC 46300, and *L. monocytogenes* ATCC 7644 used for understanding the decontamination effect of ZnO-NPs.

Preparation of ZnO-NP Solution

Nanopowder ZnO which has <50 µ particle size and 10.8 m^2/g surface area (Sigma-Aldrich 677450) diluted in tryptone soy broth (TSB, Oxoid CM0129). Nanopowder TSB solution was prepared under aseptic conditions. The stock solution was prepared in 0.05 L volume and the final concentration of ZnO-NPs were calculated 1 Molar.

Method

All our strains were stored at -20 °C before revived in TSB. Each strain was studied under optimal conditions recommended by International Organization for Standardization (ISO). For this purpose, incubation and growth parameters which were specified in ISO 6579 for *Salmonella* species, ISO 6888 for *S. aureus*, ISO 11290 for *L. monocytogenes*, and ISO 16654 for *E. coli* O157:H7 were used. The strain's growth conditions were given in Table 1.

Stock ZnO-NPs solution, which was 1 Molar concentration, diluted to 20 mmol/L in sterile TSB for investigation of the decontamination effect on target microorganisms. Later 9 ml. 20 mmol/L ZnO-

NPs solution dispensed into sterile test tubes under aseptic conditions for microbiological analysis.

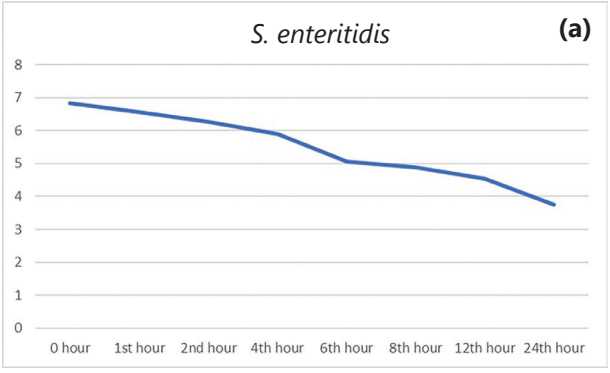
For investigation of the decontamination effect, 100 µl 24 hours old fresh cultures of the pathogens were added into sterile test tubes which were containing TSB with 20 mMolar ZnO-NPs. After inoculation of the pathogens, a counting procedure was performed using the Plate Count Agar (PCA, Oxoid CM0463) at 0, 1st, 2nd, 4th, 6th, 8th, 12th, and 24th hours. For this purpose, 9 ml serial decimal dilutions which were containing 0.1% peptone water (Oxoid CM1049), were prepared and autoclaved at 121°C for 15 minutes. Enumeration of microorganisms was executed according to ISO 4833-1 method by pour plate technic (ISO, 2014).

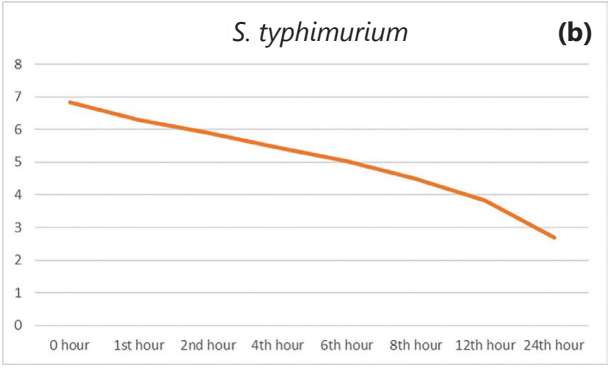
Table 1. Growth condition of test strains.

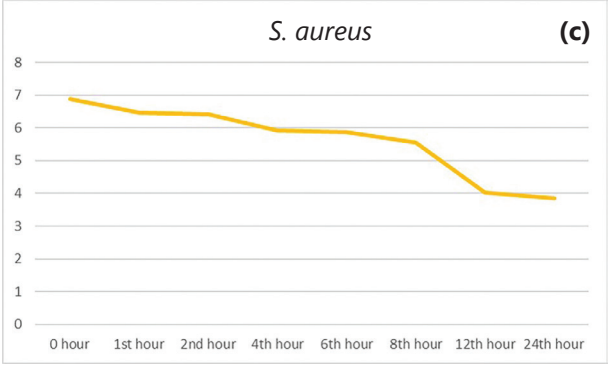
Bacteria	ID no.	Media	Opt. Temp. (°C)
Salmonella enteritidis	ATCC 31194	TSB-PCA	37
Salmonella typhimurium	ATCC 14028	TSB-PCA	37
S. aureus	ATCC 46300	TSB-PCA	37
L. monocytogenes	ATCC 7644	TSB-PCA	37
E. coli O157	ATCC 43895	TSB-PCA	37

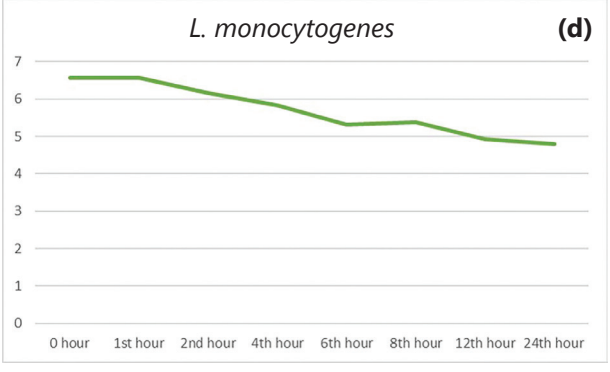
Results

In the present study, the invitro decontamination effect of 20 mmol/L concentration, <50 µ particle size zinc oxide Nanoparticles (ZnO-NPs) on important foodborne pathogens was investigated. For this purpose, five important food-borne pathogens, 3 gram-negative and 2 gram-positive were used in our study. At 0-hour, bacterial count of *Salmonella enteritidis* ATCC 31194, *Salmonella typhimurium* ATCC 14028, *S. aureus* ATCC 46300, *L. monocytogenes* ATCC 7644, *E. coli* O157 ATCC 43895 was 6.83, 6.80, 6.89, 6.56, and 6.81 Log CFU/mL, respectively. *Salmonella typhimurium* ATCC 14028 and *E. coli* O157 ATCC 43895 were found the most sensitive in all strains. On the other hand, *L. monocytogenes* ATCC 7644 was the most resistant in all tested strains. The reduction effect of 20 mmol, <50 µ ZnO-NPs were calculated 3.09, 4.14, 3.09, 1.77, and 4.08 Log CFU/mL, respectively. Analyses repeated six times and the average results shown in the figures below.









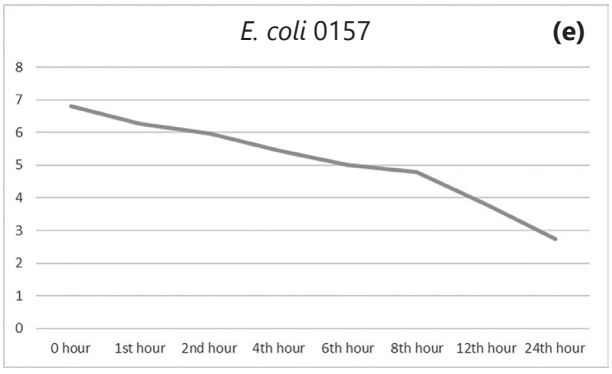


Figure 1. Decontamination effect of 20 mmol/L zinc oxide Nanoparticles (ZnO-NPs). Reduction of *Salmonella enteritidis* (a), *Salmonella typhimurium* (b), *S. aureus* (c), *L. monocytogenes* (d), *E. coli* O157 (e).

Discussion and Conclusion

In our study, the invitro decontamination effect of ZnO-NPs in 24 h was found on average 3 Log CFU/mL in all test groups. In comparison, it has been reported that gram-positive bacteria are more sensitive to ZnO-NPs than gram-negative bacteria (Tayel et al., 2011). However, in our study maximum reduction has been seen on both gram-negative and positive bacteria such as *S. aureus, Salmonella typhimurium,* and *E. coli* O157. This situation may be attributed to the fact that bacteria used in this study, were certificated strains rather than wild strains. In the present study, a considerable reduction effect was observed in all test groups with an average of 3 Log CFU/mL. Our results showed similarities with Das et al. (2017) who investigated the disinfection effect of ZnO-NPs on multi-drug resistant (MDR) strains. According to their results, a 99% disinfection effect was achieved on MDR strains. Mirhosseini and Arjmand (2014) studied the decontamination effect of ZnO-NPs against the *L. monocytogenes, E. coli, S. aureus*, and *B. cereus*. Correlatively to our results in 24 h *L. monocytogenes, E. coli,* and *S. aureus* were reduced approximately 3 or 4 Log CFU/mL. In another paper, Liu et al. (2009) were investigated the antibacterial activities of ZnO-NPs against *E. coli* O157:H7. Their results showed that ZnO-NPs dramatically inhibited *E. coli* O157:H7 strains in 24 h. The reason of this may be connected to the inhibitory effect of ZnO-NPs increased as concentration increases, and the size of the particle decreases. In another study on *Salmonella* which is an important foodborne agent, Fonseca et al. (2019) were investigated to control biofilm formation and *Salmonella* infiltration into turkey eggs by using ZnO nanocrystals. According to their results, searching for controlling microorganisms in the food hygiene area, ZnO nanocrystals were recognized as efficient, safe, reliable, and high potential alternatives by scientists. Similarly, in our study, it was determined that ZnO-NPs were highly effective on foodborne pathogens as well. In a different research, the antimicrobial effects of CuO, NiO, ZnO, and Sb_2O_3 nanoparticles were analyzed on several microorganisms such as *E. coli*, *B. subtilis*, and *S. aureus* (Baek & An, 2011). As a result, CuO nanoparticles were found to be the most effective comparing to other nanoparticles tested and followed by ZnO-NPs. It was determined that the reducing ratio of ZnO-NPs on target microorganisms was in parallel to our results. Habeeb Rahman et al. (2018) were investigated ZnO-NPs and sunlight combinations as a novel technique for *Salmonella typhimurium* disinfection in water samples. Similar to our results, it was understood that ZnO-NPs were reduced *Salmonella typhimurium* by approximately 4 Log CFU/mL. Hakeem et al. (2020) were studied ZnO-NP coated active packaging for suppressing foodborne pathogens. For this purpose, researchers have been examined the antimicrobial effects of ZnO-NPs on both gram-positive and negative bacteria such as *C. jejuni* and *Lactobacillus* on chicken meat matrix in their project. Like to our outcomes, after 3 days of storages at 4°C, the antibacterial effect of ZnO-NP coated active packaging was found more or less 3-4 Log CFU/mL. In another study examining the antimicrobial effects of ZnO-NPs on gram-positive and negative bacteria Tayel et al. (2011) were used *E. coli* O157:H7, *Salmonella enteritidis*, *Salmonella typhimurium*, and *Staphylococcus aureus* as target microorganism. Unlike our study, researchers were found *Staphylococcus aureus* the most sensitive. However, in our study *Salmonella typhimurium* and *E. coli* O157 were the most susceptible strains. These results might be connected to the concentration of ZnO-NPs in the study. It has been known that high concentration causes a high antimicrobial effect as well.

Consequently, according to the results obtained from this project ZnO-NPs have an intense decontamination effect on both gram-positive and negative foodborne pathogens. Because of nanoparticles have been identified as "Generally Regarded as Safe" (GRAS), they may be recommended to use in packaging materials, disinfectants, and solutions to extend shelf life and preserve foods for a longer time in the food industry.

Ethical Statement: This study does not present any ethical concerns.

Conflict of Interest: The authors declared that there is no conflict of interest.

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