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Photoactivated ZnO nanoparticles destroy main food pathogens *Escherichia coli* O157:H7 and *Listeria monocytogenes* $ATC_{L3}C$ 7644

K. Kairyte, Z. Luksiene
Vilnius University Institute of Applied Research
Sauletekio Ave. 10, LT-10222 Vilnius, Lithuania
E-mail: Kristina.Kairyte@tmi.vu.lt

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Food-borne diseases have been estimated to cause millions of hospitalizations and cost billions of dollars each year. This means that the existing food safety technologies cannot guarantee safe food. The aim of this study was to evaluate the antimicrobial efficiency of photoactivated ZnO nanoparticles against the food pathogens *Escherichia coli* O157:H7 and *Listeria monocytogenes* $ATC_{L3}C$ 7644.

The results have shown ZnO NPs to have a slight effect on the viability of bacteria in the dark, whereas photoactivated ZnO NPs exhibit a pronounced bactericidal activity. In certain experimental conditions, gram-negative bacteria *E. coli* and gram-positive bacteria *L. monocytogenes* were killed to undetectable level.

Summarizing, photoactivated ZnO NPs have a potential to be an effective antimicrobial tool and can be used to inactivate harmful and pathogenic microorganisms.

Key words: ZnO nanoparticles, photodestruction, antimicrobial action, *Escherichia coli*, *Listeria monocytogenes*

Introduction

Enterohemorrhagic *Escherichia coli* O157:H7 is one of the most important food-borne pathogens in the food industry and has resulted in a large number of highly publicized and expensive recalls [1]. This gram-negative bacterium can survive in acidic foods, and its infective dose is considered very low, e. g., 1 to 100 cells [2]. *E. coli* O157:H7 has been shown to survive for extended periods on stainless steel surfaces and domestic plastic cutting boards [3, 4] and has been detected on conveyor belts in beef-processing plants [5]. The gram-positive bacterium *L. monocytogenes* is a human pathogen, which is found in ready-to-eat products, such as bovine meat, pork meat, poultry, cheese, and fishery products [6]. A total of 1.601 confirmed cases of listeriosis were reported in 26 EU Member States with a high fatality rate of 17 % among the cases in 2010 [7].

Since the existing conventional chemical antibacterial treatments used to combat food pathogens are not effective enough, the development of new antimicrobial agents has been attracting increasing attention. Moreover, the usage of chemical sanitizers is suspected to be environmentally unsound as it is associated with occupational and operational hazards and is potentially harmful for humans [8]. Consequently, the development of nonthermal and nonchemical sterilization methods seems promising.

Nanoparticles of metal oxides represent a new class of important materials that are increasingly being developed for usage in research and health-related applications [9, 10, 11], including food safety [12]. Recently, many various nanoparticles have been synthesized and tested for their antimicrobial activity against different food-borne bacteria. The vast majority of these nano-sized agents are metal nanoparticles due to their small size, large surface-to-volume ratio, chemically alterable physical properties, ability to change chemical and physical properties with respect to size and shape, etc. [13]. The nanoparticles that have been tested for their antimicrobial activity are silver oxide [14], titanium dioxide [15], zinc oxide [16, 17], nickel and copper oxides [18], magnesium oxide nanoparticles [19], iron nanoparticles [20], carbon-based nanomaterials [21], etc. Metal oxides such as zinc oxide, titanium dioxide, and magnesium oxide are not only stable but also generally regarded as safe to human beings [16]. However, there are still very few studies on the evaluation of photoactivated ZnO NPs efficiency against food pathogens.

The aim of this study was to evaluate the antimicrobial efficiency of photoactivated ZnO nanoparticles against the food pathogens *Escherichia coli* O157:H7 and *Listeria monocytogenes* $ATC_{L3}C$ 7644.

Materials and methods

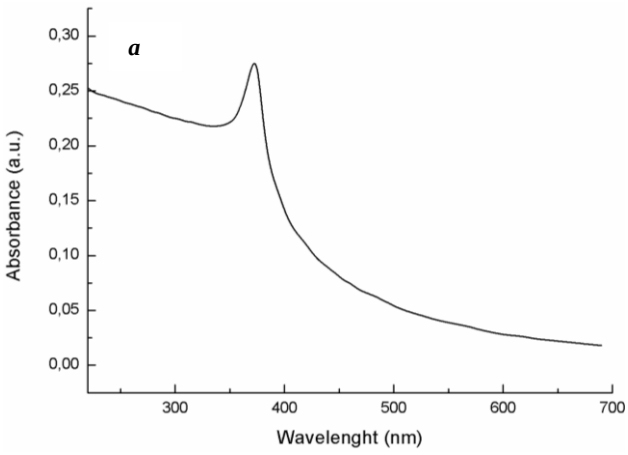
Pure cultures of E. coli O157:H7 and Listeria monocytogenes $ATC_{L3}C$ 7644

Two food pathogens were used for experiments: *L. monocytogenes* $ATC_{L3}C$ 7644 and *E. coli* O157:H7. All bacteria were maintained at 37 ºC for 24 h onto Luria-Bertani Agar (LBA; Liofilchem, Roseto degli Abruzzi, Italy). *Listeria* and *Escherichia* cultures were grown overnight (~16 h) at 37 ºC in 20 ml of tryptone soya medium supplemented with 0.6 % yeast extract (TSYE) (Liofilchem) and in 20 ml of Luria-Bertani medium (LB; Liofilchem), respectively, with agitation of 120 rev/min (Environmental Shaker – Incubator ES – 20; Biosan, Riga, Latvia). Afterwards, *Listeria* and *Escherichia* bacterial cultures were diluted 20 times with the fresh TSYE and LB medium (OD_{540} = 0.164), respectively, and grown at 37 ºC

in a shaker (120 rev/min) to the mid-log phase (~1.16×10^9 colony-forming units (CFU/ml), OD_{540} = 0.9 for *Listeria*; ~1×10^8 CFU/ml, OD_{540} = 0.9 for *Escherichia*). The bacterial optical density was determined in a 10.01 mm cuvette at λ = 540 nm (Helios Gamma & Delta spectrophotometers; ThermoSpectronic, Waltham, MA, USA). Cells were then harvested by centrifugation (10 min, 3420×g) (Mikro 200, Hettich Zentrifugen, Germany) and resuspended in 0.9 % NaCl to ~1×10^7 CFU/ml and used for the further experiments.

Inactivation of E. coli O157:H7 by photoactivated ZnO NP

Aliquots (20 ml) of bacterial suspension (1×10^7 CFU/ml in 0.9 % NaCl) with an appropriate concentration of ZnO NPs (1×10^{-5}–1×10^{-3} mol/l) were incubated in the dark at 37 ºC. For the following experiments, the cells were incubated in a shaker (130 rev/min) for various periods (10–60 min). Afterwards, 150 µl aliquots of a bacterial suspension were withdrawn, placed into sterile flat bottom wells and exposed to light for 30 min. A LED-based light source for photosensitization experiments (λ = 400 nm; intensity – 9.6 mW/cm^2) was constructed in our institute (Fig. 1). The light dose delivered to the sample was calculated as light intensity multiplied by irradiation time.



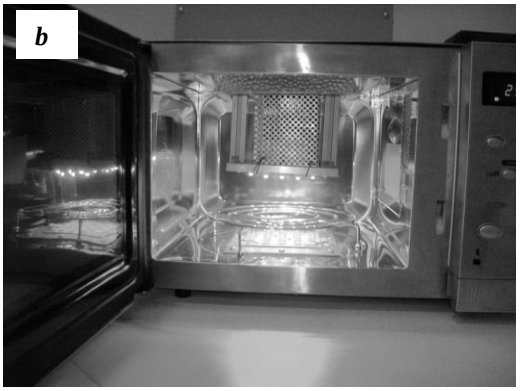


Fig. 1. Absorption spectrum of 1×10^{-4} mol/l ZnO NPs in NaCl (*a*) and a LED-based light source device constructed in our institute (*b*). Absorption spectrum was recorded in a 10.01 mm cuvette using a Helios Gamma spectrophotometer

Bacterial cell survival assay

The antibacterial effect of photoactivated and non-photoactivated ZnO NPs on bacterial cells was evaluated by the spread plate method. Thus, 100 µl of appropriate dilutions of a bacterial test culture after treatment was surface-inoculated on the LBA plates. Afterwards, the bacteria were incubated in a thermostat for 24 h at 37 ºC. The surviving cell populations were enumerated and expressed as $\log_{10}$ (CFU/ml).

Results and discussion

Antimicrobial photosensitization-based treatment has a number of advantages in comparison with traditional anti-microbial tools, since it is cost-effective, environmentally friendly and does not induce appearance of resistant bacterial forms. Moreover, bacterial susceptibility to singlet oxygen and other radical products of photo-sensitized reactions is very high [10].

The obtained data indicate that ZnO nanoparticles at a concentration of 1×10^{-3} mol/l and incubation time 60 min without photoactivation are ineffective and can diminish the *E. coli* population insignificantly (~1.5 log). Lower ZnO NPs concentrations (1×10^{-5}, 5×10^{-5}, 1×10^{-4}, 5×10^{-4} mol/l) were not toxic to the investigated bacteria, either, since a slight effect on their viability was found in the dark (0–1 log reduction) (Fig. 2). Similar response patterns to ZnO NPs treatment was shown by *Listeria* cells. The incubation of culture cells with 1×10^{-5}–1×10^{-3} mol/l ZnO NPs had no effect on bacterial growth (Fig. 3). Only incubation of both bacteria with ZnO NPs and the following illumination with 400 nm light resulted in a reduction of the bacterial count.

There are some studies to show a strong antibacterial effect of ZnO NPs against *E. coli* using a much longer incubation time. For example, Zhang et al. [22] have found that ZnO suspension at a concentration of 1.25×10^{-3} mol/l leads to ~10^5 reduction of bacterial count after 24 h of incubation time. Liu with colleagues [12] have shown a complete inhibition of *E. coli* growth after 24 h of incubation with 1.2×10^{-2} mol/l ZnO NPs. Jin et al. [23] reported ~2 log reduction of *L. monocytogenes* after 48 h of incubation with 10 mg/ml ZnO quantum dots as compared with the control. The results of our study are in line with the latter results as the 1h incubation of bacterial cells with 1×10^{-5}–1×10^{-3} mol/l ZnO NPs had a slight effect on their viability (0–1.5 log reduction).

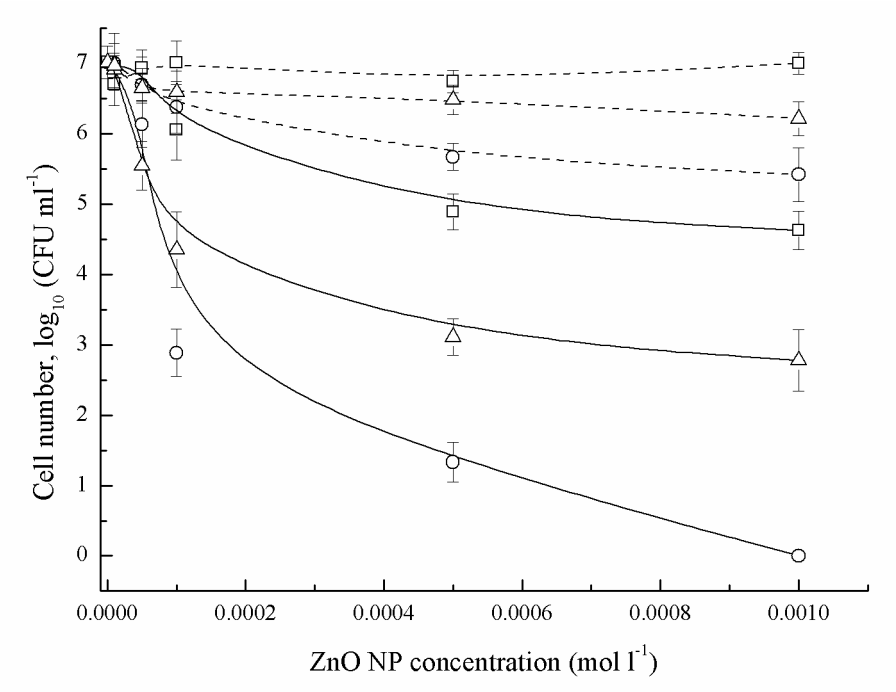


Fig. 2. Inactivation of *E. coli* O157:H7 by photoactivated (solid line) and not-photoactivated (dash line) ZnO NPs as a function of the used concentration: (- - □ - - , —□—) 10 min incubation, (- - △ - - , —△—) 30 min incubation, (- - ○ - - , —○—) and 60 min incubation. Each point is the average of 3–6 experiments, and error bars sometimes are too small to be more visible

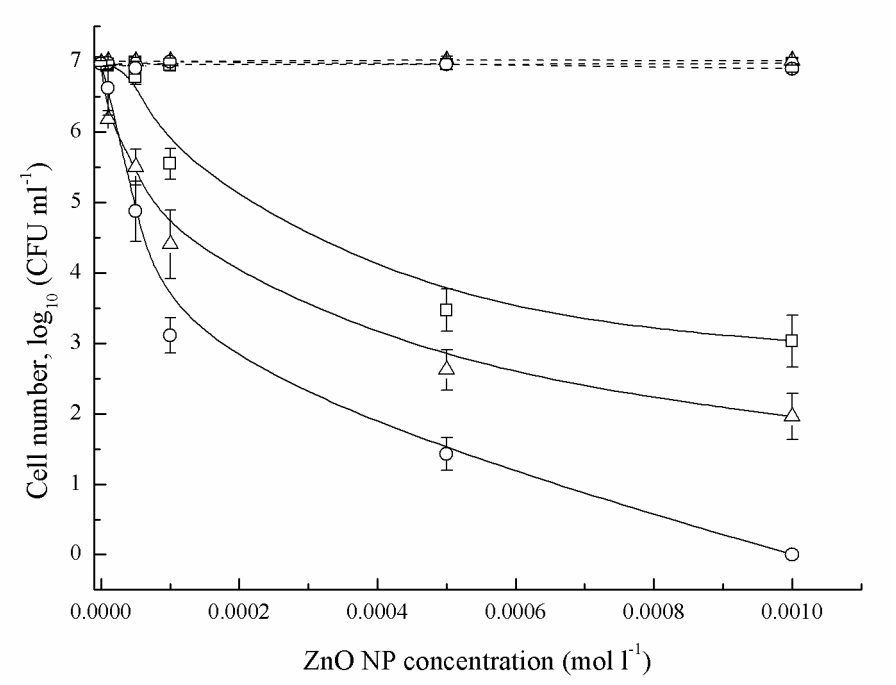


Fig. 3. Inactivation of *L. monocytogenesi* ATCL$_3$C 7644 by photoactivated (solid line) and non-photoactivated (dash line) ZnO NPs as a function of the used concentration: (- - □ - - , —□—) 10 min incubation, (- - △ - - , —△—) 30 min incubation, (- - ○ - - , —○—) and 60 min incubation. Each point is the average of 3–6 experiments, and error bars sometimes are too small to be more visible

Our results indicate that photoactivation of ZnO with visible light (λ = 400 nm; 17.3 J/cm^2) enhances the antimicrobial action of ZnO nanoparticles against both bacteria studied. The antibacterial activity increases with increasing nanoparticle concentration and incubation time (0–60 min). A remarkable decrease of microbial counts (from 7 log in control to the undetectable level in treated ones) was observed as soon as after 60 min of incubation with 1×10^{-3} mol/l ZnO and at 17.3 J/cm^2 illumination dose.

Data presented in Fig. 3. have revealed *Listeria* to be more sensitive to photoactivated ZnO NPs as a lower concentration of photosensitizer causes a higher inactivation of *Listeria as* compared to *Escherichia* cells. There is no obvious evidence of a greater susceptibility of gram-positive or gram-negative bacteria to ZnO NPs treatment. Some researchers have reported a higher sensitivity of gram-positive bacteria to ZnO NPs treatment in comparison with gram-negative bacteria [16, 24, 25]. On the contrary, gram-positive *Lactobacillus helveticus* bacteria were more resistant to the treatment with ZnO activated with ultraviolet light as compared to gram-negative *E. coli* [26]. The authors suggest that the different susceptibility of the bacteria could be related to the differences in cell wall structure, cell physiology, metabolism, degree of contact or surface charge of a bacterial cell.

Moreover, nanoparticle–bacteria interactions depend not only on the type and physiological state of the bacteria, but also on the physicochemical properties of the nanoparticles. The antimicrobial activity of nanoparticles may be related to several mechanisms. First, the nanoparticles can either directly interact with the microbial cells, e.g., by interrupting transmembrane electron transfer, disrupting / penetrating the cell envelope, or oxidizing cell components. Second, NPs can produce secondary products (e.g., reactive oxygen species (ROS) or dissolved heavy metal ions) causing cell destruction [27].

In summary, the results of this study have shown a high antimicrobial efficacy of photoactivated ZnO NPs against gram-positive bacteria *L. monocytogenes* and gram-negative *E. coli*. This implies that ZnO NPs could be potentially used as an effective antibacterial agent of food safety. In addition, further studies should be performed to understand the pathways and mechanisms of cell destruction after photoactivated ZnO NPs treatment.

Conclusions

The antimicrobial activity of ZnO nanoparticles without light exposure is insignificant against *E. coli* and *L. monocytogenes in vitro.* Photoactivation (λ = 400 nm) of nanoparticles enhances remarkably their antimicrobial activity: in certain experimental conditions, both gram-negative bacteria *Escherichia coli* O157:H7 and gram-positive bacteria *Listeria monocytogenes* $ATCL_3C$ 7644 were killed to undetectable levels.

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K. Kairytė, Ž. Lukšienė

FOTOAKTYVIOS ZnO NANODALELĖS PAGRINDINIŲ MAISTO PATOGENŲ *ESCHERICHIA COLI* O157:H7 IR *LISTERIA MONOCYTOGENES* $ATC_{L3}C$ 7644 NAIKINIMUI

S a n t r a u k a

Dėl per maistą plintančių ligų kasmet gydoma milijonai užsikrėtusių žmonių, kovai su šiomis ligomis išleidžiami taip pat milijonai JAV dolerių. Tai rodo, kad esamos maisto produktų apdorojimo technologijos neužtikrina saugaus maisto. Todėl šio tyrimo tikslas buvo įvertinti fotoaktyvių ZnO nanodalelių poveikį *Escherichia coli* O157:H7 ir *Listeria monocytogenes* ATCL3C 7644 patogenams.

Gauti rezultatai parodė, kad šviesa nepaveiktos ZnO nanodalelės turi silpną poveikį bakterijų gyvybingumui, o fotoaktyvios ZnO nanodalelės pasižymi ypač stipriu antibakteriniu poveikiu. Šio tyrimo sąlygomis *E. coli* ir *L. monocytogenes* bakterijas pavyko visiškai inaktyvuoti.

Apibendrinant galima teigti, kad fotoaktyvios ZnO nanodalelės pasižymi efektyviomis antimikrobinėmis savybėmis, kurias naudojant įmanoma sunaikinti pavojingus patogeninius mikroorganizmus.