Antibacterial activity and mechanism of ZnO nanoparticles on *C. jejuni*

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ZnO

- It is stable under high temperatures and pressures, which makes them suitable for use in harsh food processing conditions.
- ZnO is listed as “Generally Recognized as Safe” to human beings by FDA.
- ZnO has been incorporated into some packaging materials to preserve colors and prevent spoilage of food.
- As a food additive, ZnO is the most commonly used zinc source in the fortification of cereal-based foods.
Scanning electron microscope images of ZnO nanoparticles

Spherical shaped ZnO nanoparticles with average diameter of 50 nm.

Panchakarla et al. 2007
ZnO nanoparticles

- ZnO nanoparticles have a broad spectrum of antibacterial activities on both G⁺ and G⁻ bacteria, including *E. coli O157:H7*, *L. monocytogenes*, *S. Enteritidis*, *B. subtilis*, *S. aureus*, *P. aeruginosa*, and *E. faecalis*.

- ZnO nanoparticles have significantly higher antibacterial effect on *S. aureus* than other metal oxide nanoparticles (MgO, TiO2, Al2O3, CuO, and CeO2).

- The antibacterial activity of ZnO is dependent on the size and concentration of the particles.

- Nano-sized particles of ZnO have more pronounced antimicrobial activities than large-size particles due to small size and high surface-to-volume ratio.

- The interaction between ZnO nanoparticles and *E. coli* resulted in disruption of cell membrane integrity.
Scanning electron microscope images of *E. coli* O157:H7

ZnO nanoparticles

Liu *et al.* 2009
Background on *Campylobacter* spp.

- Gram negative
- Spiral shaped rods
- Highly motile
- Thermophilic
- Microaerophilic
Background on *Campylobacter* spp. (continue…)

- *Campylobacter* is a leading bacterial cause of human gastroenteritis in the world.
- Consumption of *Campylobacter*-contaminated food and water is the common cause of the infection.
- The contamination rate of *Campylobacter* in poultry products can be as high as 80%.
- Among 18 different species, *C. jejuni* is responsible for over 90% of the *Campylobacter* infections.
- *C. jejuni* is extremely sensitive to oxidative stress due to the lack of some important oxidative stress response genes (*soxRS* and *oxyR*) and a global stationary-phase stress response gene (*rpoS*).
Growth inhibition of *C. jejuni* by ZnO nanoparticles

- Both on agar plates and in broth, the growth of *C. jejuni* was completely inhibited at ≥ 0.03 mg/ml of ZnO nanoparticles.
- The concentration of the nanoparticles for *E. coli* O157:H7 growth inhibition was 8-32 times higher than that for *C. jejuni*.
- ZnO nanoparticles function as a bactericidal, not bacteriostatic agent on *C. jejuni*.
- The MIC for *C. jejuni* was 8-16 fold lower than *E. coli* O157:H7 and *Salmonella*.

<table>
<thead>
<tr>
<th>Strains</th>
<th>MIC (mg/ml)</th>
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<tbody>
<tr>
<td><em>C. jejuni</em></td>
<td>0.025-0.05</td>
</tr>
<tr>
<td><em>E. coli</em> O157:H7</td>
<td>0.4</td>
</tr>
<tr>
<td>S. Enteritidis</td>
<td>0.4</td>
</tr>
</tbody>
</table>
Survival of *C. jejuni*, *Salmonella*, and *E. coli* O157:H7 exposed to ZnO nanoparticles
Scanning electron microscopic images of *C. jejuni*

A

B

ZnO nanoparticles + -

1.0μm
Principle of EMA-qPCR

Viable cells

Membrane compromised cells

Photo cross-linking

Real-time PCR

Reduced or no amplification

• Ethidium monoazide
EMA-qPCR analysis of *C. jejuni* membrane permeability

The treatment of ZnO nanoparticles on *C. jejuni* increased cell membrane permeability.
Relative gene expression levels of ZnO nanoparticle treated/untreated *C. jejuni*

Two oxidative stress genes (*katA* and *ahpC*) and one general stress gene (*dnak*) were up-regulated 52, 7, and 17 fold, respectively, suggesting the antibacterial mechanism is likely due to the induction of oxidative stress in *C. jejuni* cells.
Summary

- ZnO nanoparticles had remarkable antibacterial activity and a lethal effect against *C. jejuni*, even at low concentrations.
- The MIC for *C. jejuni* was 8-16 fold lower than that for *E. coli* O157:H7 and *Salmonella*.
- In *C. jejuni*, ZnO nanoparticles induced:
  1. Significant morphology changes
  2. Measurable membrane leakage
  3. Substantial increases (up to 52 fold) of oxidative stress gene expression
- The antibacterial mechanism of ZnO nanoparticles:
  1. Direct interaction between ZnO nanoparticles and cell surfaces affects cell membrane permeability;
  2. Then the nanoparticles enter and induce oxidative stress in bacterial cells;
  3. Which results in the inhibition of cell growth and eventually cell death.
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