Characterization of Antibacterial Mechanisms of Zinc Oxide in *Staphylococcus aureus*

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Background

Proposed Mechanisms of ZnO NP Antibacterial Activity

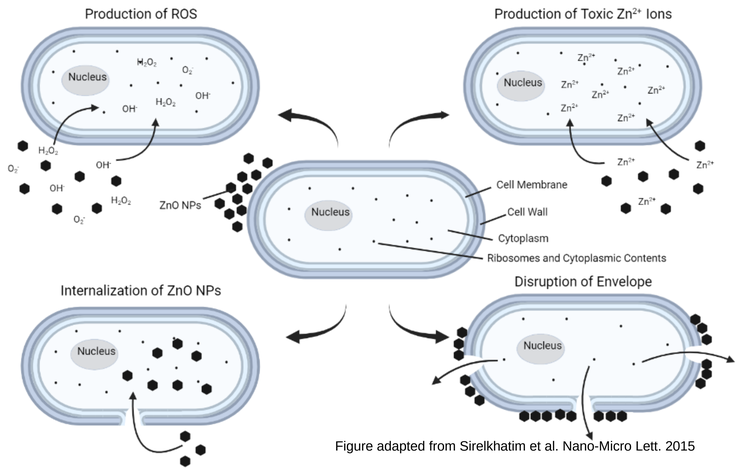
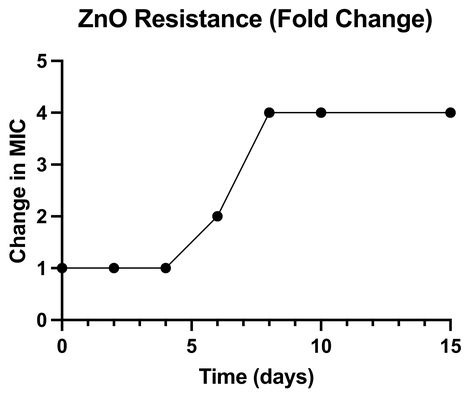


Figure adapted from Sirelkhatim et al. Nano-Micro Lett. 2015

Literature suggests that the mechanism of ZnO antibacterial action could occur through internalization of the ZnO that results in disruptions in cellular function, production of ROS, production of Zn^{2+} ions and other toxic ionic complexes, and loss of cellular integrity due to contact or interactions between ZnO surfaces and the cell envelope. Previous work (Reeks et al. 2021) has shown that internalization of ZnO NPs is not necessary for ZnO killing, so we chose to investigate the other mechanisms.

ZnO Resistance



At day 0, the MIC of ZnO was 1.25 mg/ml. After 8 days, of passing the *S. aureus* at sublethal conditions, we observed a 4-fold increase in the MIC (from 1.25 mg/ml to 5 mg/ml).

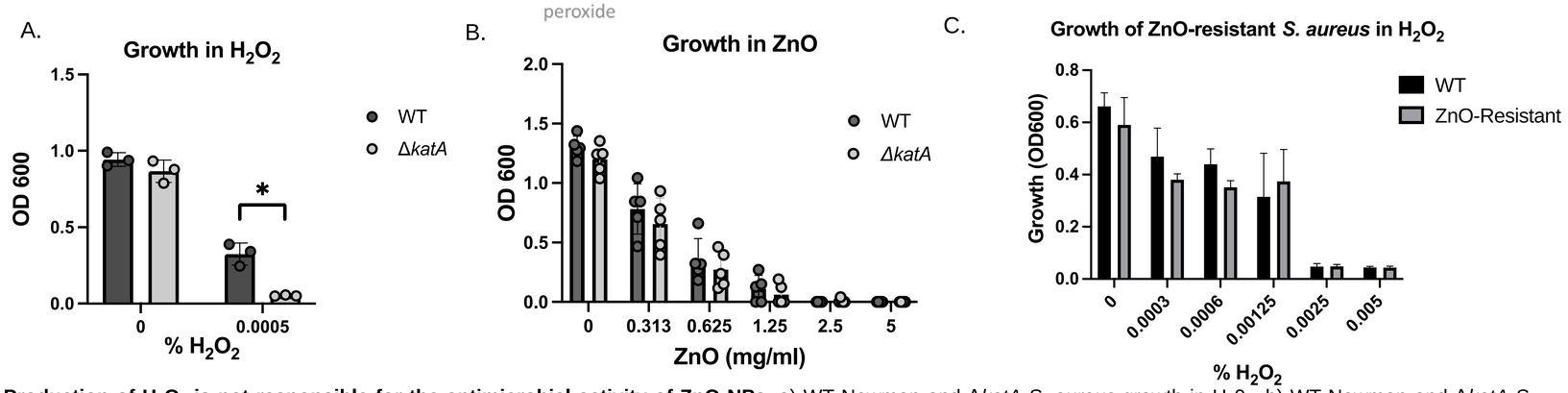
Comparison of the MICs of different ZnO NP sources with wild type and ZnO Resistant *S. auerus*

Particle Type	Wt MIC (mg/ml)	ZnO^R MIC (mg/ml)
Sigma Aldrich	1.25	5
Alfa Aesar	1.25	5
ZoChem	2.5	10

Role of H_2O_2

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

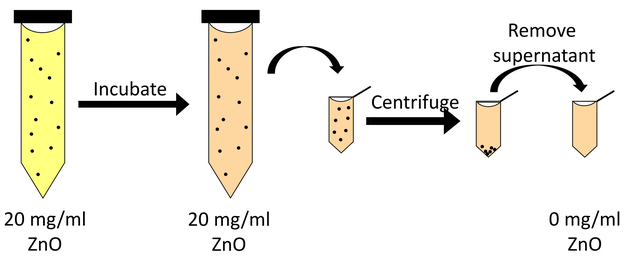
Hydrogen peroxide → Water + Oxygen

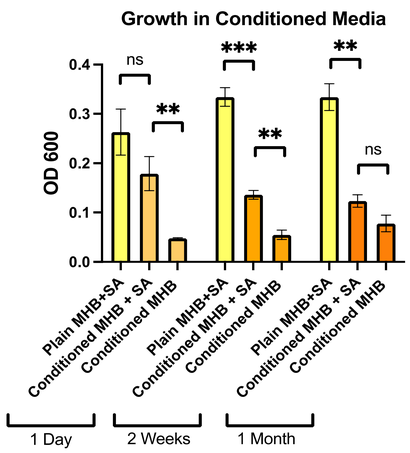


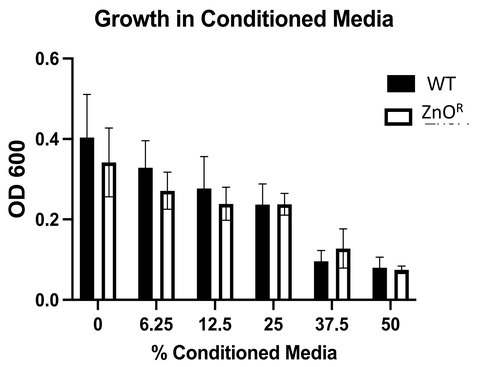
Production of H_2O_2 is not responsible for the antimicrobial activity of ZnO NPs. a) WT Newman and Δ*katA S. aureus* growth in H_2O_2. b) WT Newman and Δ*katA S. aureus* growth in Sigma-Aldrich NPs. c) WT Newman and ZnO Resistant *S. aureus* in H_2O_2. a-c) Error bars represent mean +/- SD of at least three independent trials. **p < 0.01 as determined by unpaired t-test.

Role of Physical Contact

Method:



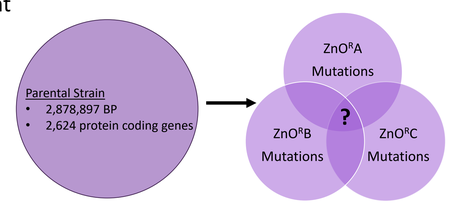




Media conditioned with ZnO NPs retains ability to inhibit bacterial growth after removal of NPs Growth of *S. aureus* was measured in normal MHB and conditioned media. Conditioned media without *S. aureus* acted as a blank. Data are presented as mean ± SD and assays were repeated at least three independent times. Different letters represent statistically significant differences (*p < 0.05, **p<0.01) as determined by one-way ANOVA.

Conclusions and Future Directions

1. Attempt to generate a *S. aureus* strain that is resistant to ZnO conditioned media
2. Evaluate the role of Zn^{2+} using cytochrome c assay to measure cell charge
3. WGS of Independent ZnO^R Strains



References

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