Recombinant, antibiotic-delivering probiotics for use in agriculture

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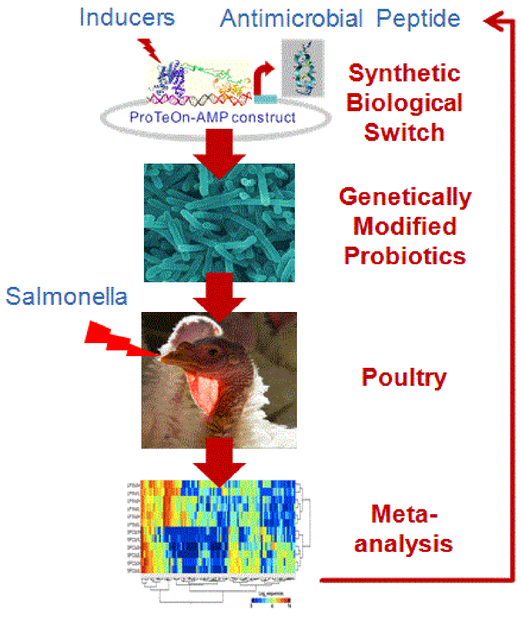
Challenge

An estimated 50-70% of antibiotics produced in the United States are administered to cattle, pigs and poultry at sub-therapeutic levels to improve growth and feed efficiency. This practice creates a potentially vast reservoir for the selection of drug-resistant bacteria.

Proposed Solution

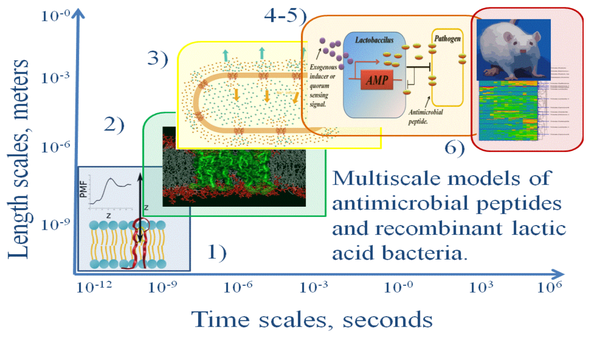
Engineer lactic acid bacteria (LAB) to produce antimicrobial peptides (AMPs) when infection occurs. Use modified organisms as antibiotic substitutes.

- AMPs are proteins with remarkable antibiotic properties.
- AMPs cannot be administered directly because as proteins they will be quickly degraded by the host.
- LAB, also known as probiotics, can be safely administered to humans and animals.
- LAB can be genetically modified to express AMPs in a controllable manner.
- Focus on poultry, important sources of protein in diets worldwide. because Minnesota is the top turkey producing and processing state in the US.



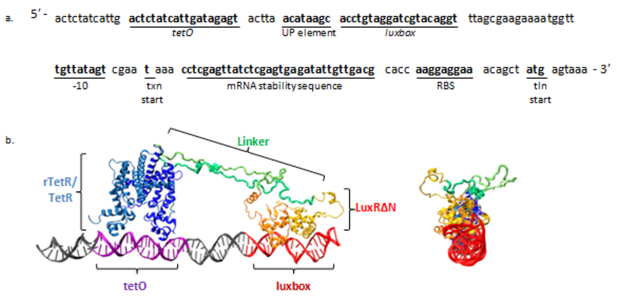
Computer-Aided Design of AMPs

❑ Multiscale models of antimicrobial peptides.



- Explain their mechanism of action and guide the design of new sequences.
- Free energy calculations of peptide-membrane binding, peptide aggregation and pore formation.
- Molecular dynamics simulations of peptide pore and ion transport.
- Poisson-Nernst-Planck equations used to model electrodiffusion of ions through pores.
- Whole cell models couple ion transport, transmembrane potential collapse, osmotic pressure, water transport and cell volume.

Computer-Aided Design of Synthetic Biological Constructs

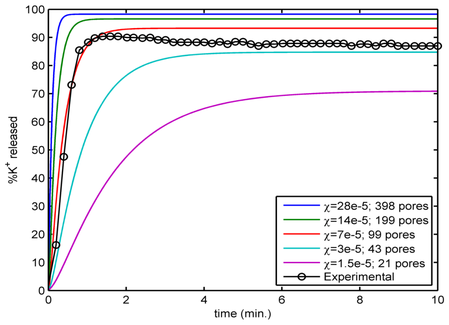


- Multiscale models of protein-DNA devices to control the expression of AMPs.
- Molecular models of DNA promoters and transcription factors..
- Stochastic models of gene regulatory networks.

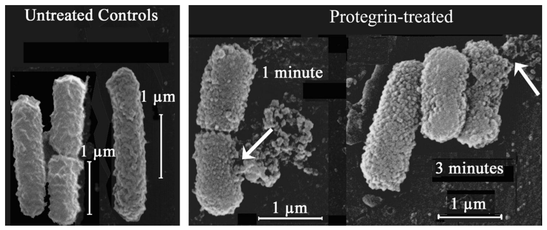
In vitro Experiments

❑ Antimicrobial peptide activity tests against bacteria.

- *E. coli* ML-35p organisms grown in 100 mM NaCl were treated with various concentrations of protegrin-1 (PG-1)

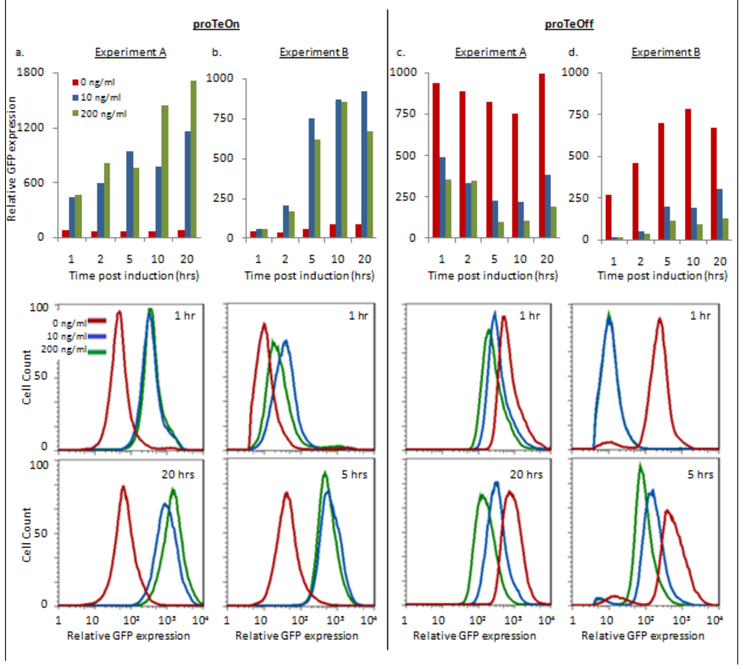


K⁺ release behavior
The experimental data (open circles) corresponds to the result of treating exponential-phase E. coli with 25 µg/mL of PG-1. Solid lines represent modeling results.



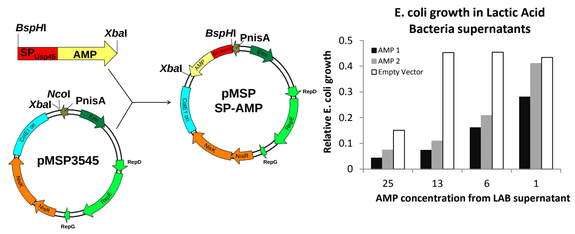
❑ Synthetic, protein-based, regulatory devices.

- *E. coli* were engineered to express green fluorescence protein under the control of anhydrous tetracycline.



❑ Synthetic, inducible antimicrobial peptide expression systems.

- We use the pMSP3545 plasmid to carry promoter libraries and AMP sequences in lactic acid bacteria.



- E. coli growth was inhibited by up to 80% upon treatment with recombinant lactic acid bacteria supernatants expressing AMP_1. The greatest inhibition was observed after serial dilutions (down to 5-15%) of the concentrated supernatant. The E. coli growth profiles were very similar upon treatment with the supernatant from different clones.

Animal Model Tests

❑ Supply engineered probiotics to poultry and complete the following investigations:

- Determine whether the probiotic bacteria reside in the bird GI tract.
- Assess how probiotic bacteria modulate the gut microflora.
- Verify that poultry challenged by *Salmonella* fair well when on a probiotic diet.

References

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Acknowledgements

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