COLLEGE OF SCIENCE
THE UNIVERSITY OF ALABAMA IN HUNTSVILLE

Research Horizons Day & Research Week March 11-15, 2019

Research Horizons Day

Functional cell separation of transformable microorganisms from complex microbial niches

Tamara Zaza[1], Shubha RL Malla[2], Shannon E Weigum[2], Tatyana Sysoeva[1]
[1]Department of Biological Sciences, UAH; [2]Department of Biology, TSU

Background

Antibiotic resistance is a global challenge in medicine today, rendering routine bacterial infections increasingly difficult to treat. Natural transformation is the ability of bacteria to take up extracellular DNA from its surroundings and integrate this genetic material into its own genome, allowing for fast acquisition of new traits, such as antibiotic resistance.

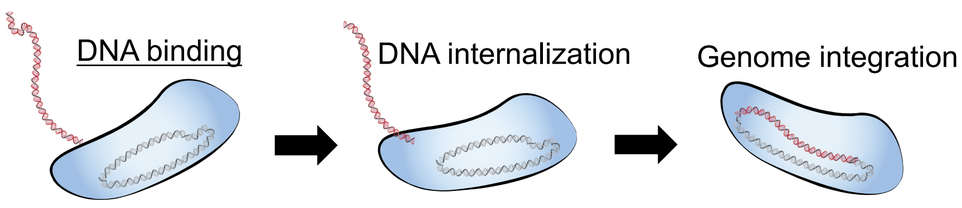

Fig. 1. *Acquisition of antibiotic resistance via natural transformation.*

It is unknown how many bacteria in total are capable of natural transformation and are contributing to the spread of antibiotic resistance through this route. We set to develop methods of isolation of novel transformable microorganisms.

Approach

All naturally transformable bacteria must first bind extracellular DNA, and we aim to use this property to isolate competent organisms from complex mixtures. We used *Bacillus subtilis,* a known transformable bacterium, to develop our method.

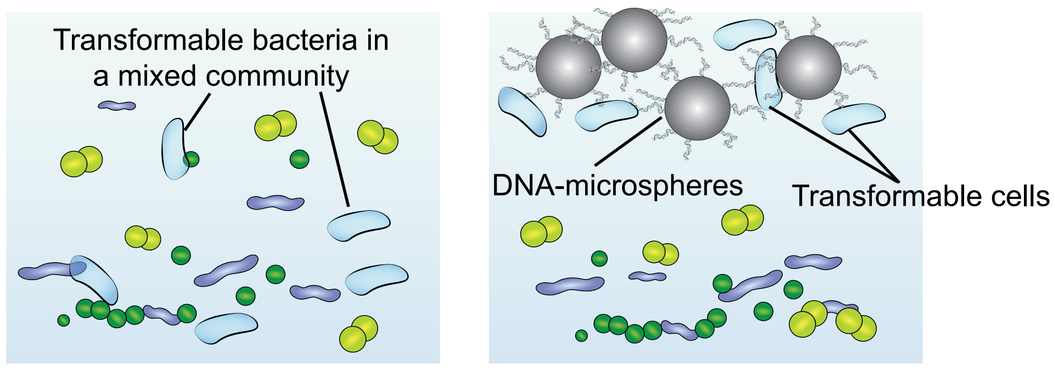

Fig. 2. *Functional cell separation from complex microbial mixtures (left) via binding of the competent cells (blue) to DNA attached to buoyant microspheres (right).*

Results

- We designed two DNA fragments of different sizes suitable for transformation, and produced them via PCR amplification.

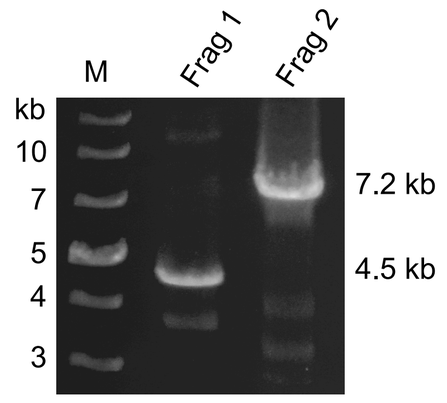

Fig. 3. *Gel electrophoresis shows the two designed linear DNA fragments (4.5 and 7.2 kb) were obtained by PCR amplification from a larger plasmid.*

- We tested that *B. subtilis* cells can be transformed with these fragments and quantified the efficiency of transformation. The results suggest that a longer DNA fragment transforms cells more efficiently.

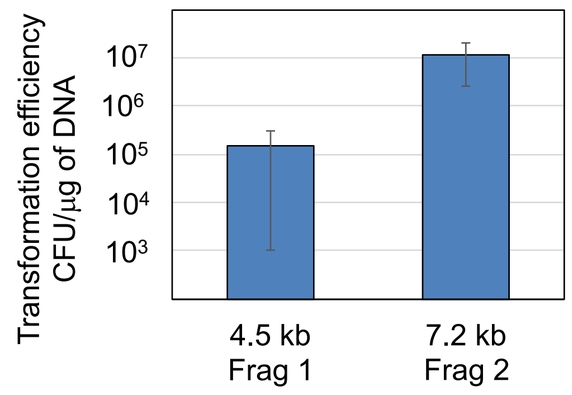

Fig. 4. *Transformation efficiencies of B. subtilis with obtained DNA fragments.*

- Silica microspheres were derivatized with covalently attached, fluorescently-labeled (Cy5) DNA.

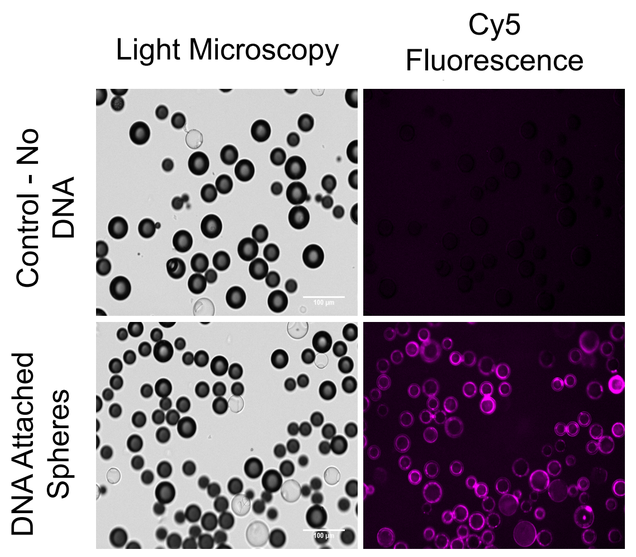

Fig. 5. *Attachment of the DNA to the epoxy-coated silica microspheres via the amine group of the Cy-5 modified oligonucleotide.*

Conclusions and Future Work

- In summary, we designed and prepared two DNA fragments that can efficiently transform *B. subtilis* bacteria. We also successfully attached DNA to the silica microspheres in preparation for cell isolation.
- These results will be used to test whether the competent fraction of the *B. subtilis* cells can be isolated via separation with the DNA-bound microspheres.
- If successful, this method will be applied to complex mixtures of microorganisms, such as those found in the soil or gut microbiome, to seek novel transformable bacteria.

Acknowledgements

This research was supported by the UAH startup funds to T.A.S. Special thanks to my lab members for their help and support!

TEXAS STATE UNIVERSITY®

HONORS COLLEGE
THE UNIVERSITY OF ALABAMA IN HUNTSVILLE