Effect of DNA Format on *Bacillus subtilis* Transformation Efficiency

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INTRODUCTION

Medicine is undergoing reform in a variety of aspects, including the growing presence of proteins in pharmaceuticals. Currently, Escherichia coli is used most often as a protein production factory because of its genetic accessibility, despite it being neither the most efficient nor the highest quality for pharmaceutical purposes (Westers et al. 2004). As the need for efficient and safe pharmaceutical protein production grows, the need for a more suitable, scalable host for protein production proves vital.

The bacterial species Bacillus subtilis has substantial potential to serve as an efficient protein production host. It poses multiple advantages over E. coli: unlike E. coli, it is a GRAS—generally recognized as safe—organism; it is naturally capable of secreting proteins at a high rate; and it could house better conditions for protein folding (Liu et al. 2013).

However, transforming B. subtilis with foreign DNA is notoriously difficult (Jakobs 2015), due to properties like its thick cell wall and innate genetic defense system (Claverys 2009; Jakobs 2015; Kruger 2011). Thus, two research questions were proposed—whether transformation is more efficient using multimeric DNA rather than monomeric DNA, and then, how the length of multimeric DNA affects efficiency.

DATA AND FINDINGS

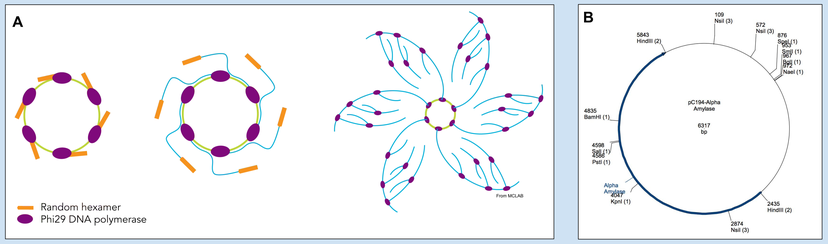

Fig. 1: Schematic diagram of the RCA process (**A**). Random hexamer primers anneal to the circular template DNA at multiple sites. Phi29 DNA polymerase extends each of these primers to synthesize new DNA. The process continues, resulting in exponential, isothermal amplification. Plasmid pC194 with Amylase was used in this study (**B**). Nsi1 restriction enzyme sites were indicated.

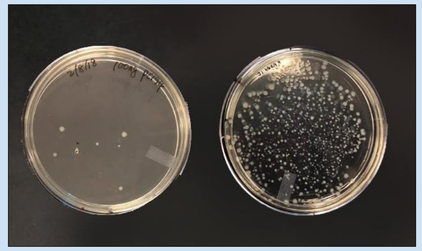

Fig. 2. *Bacillus* Transformation with RCA product. Colonies in left plate were transformants with the positive pC194 control (DNA monomer) while in right plate, with RCA product—multimeric DNA.

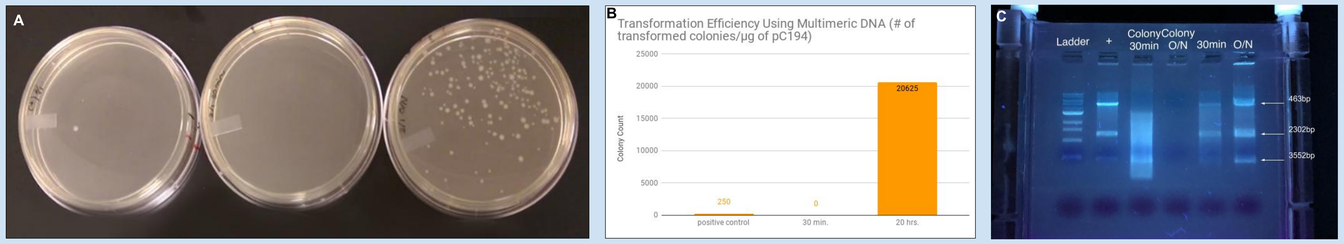

Fig. 3. Effect of DNA multimer length on transformation. From left to right plate (**A**) were the bacillus transformants with DNA monomer (plasmid pC194 control), short DNA multimer (30min RCA product) and long DNA multimer (20hr RCA product), respectively. Bar chart denoting transformation efficiencies of the plates (**B**). The length of RCA products was examined by Nsi1 restriction enzyme digestion in agarose gel (**C**).

CONCLUSIONS, IMPLICATIONS, AND NEXT STEPS

Using RCA to amplify target DNA is a viable choice for making transformation of *Bacillus subtilis* more efficient, potentially saving millions of dollars in the R&D of pharmaceutical proteins, thus making drugs more affordable and accessible.

Even so, the experiment was slow and manual, so companies would need to scale up the RCA and transformation process with greater automation and efficiency.

The lab was also prone to procedural variation. For example, the temperature of the incubator would fluctuate, which could have killed cells and affected the transformation efficiency. This experiment should be repeated in a consistent lab environment with more trials to confirm findings. Experiment 2 should also be repeated with more time points in order to find the period that would result in the optimal transformation efficiency.

Bacterial species vary in structure, so the results for *B. subtilis* cannot simply be applied to other species. Testing the process on other bacteria can widen the range of pharmaceutical proteins that can be produced more efficiently.

RESEARCH METHODOLOGIES

First Experiment: Compare the transformation rate of *B. subtilis* of DNA monomer (plasmid form) and DNA multimer (RCA product)

1. Amplify DNA plasmid through rolling circle amplification (RCA)
2. DNA monomer transformation (using plasmid)
3. DNA multimer transformation (using RCA product)
4. Compare transformation efficiency of both

Second Experiment: Effect of length of DNA multimer on the transformation rate of *B. subtilis*

1. Prepare two different lengths of DNA multimer by stopping the RCA reaction at 30 minutes and overnight
2. DNA multimer transformation for both 30-minutes and overnight RCA product
3. Compare transformation efficiency of both
4. Use gel electrophoresis to compare the lengths of DNA multimer
5. Use gel electrophoresis to confirm that DNA multimer is longer than DNA monomer

DISCUSSION, ANALYSIS, AND EVALUATION

The results of Experiment 1 (Figure 2) shows that the plate with cells transformed with multimeric RCA product yielded approximately 512 colonies, whereas the plate with cells transformed with monomer plasmid (positive control) yielded six colonies. With a transformation rate 85 times greater than the positive control, multimeric DNA produces a higher transformation efficiency (TE) than monomeric DNA in *B. subtilis.*

Continuing with Experiment 2 (Figure 3), where RCA products of differing timepoints were generated, the effect of DNA multimer length on TE was assessed. The longer the RCA reaction was left to run, the greater the DNA multimer length. Figure 3c verifies that the RCA products were of the correct DNA fragments, as the band sizes match those of the control pC194. It also shows that overnight (O/N) RCA (approx. 20hr generation) was concentrated with more DNA than the 30min RCA, as the O/N bands are more solid and bright. This observation is affirmed in the plates themselves (Figure 3a)—the O/N RCA had a transformation efficiency of 20625 transformants/µg plasmid while the 30min RCA had 0 transformants/µg plasmid. While the 30min RCA should have hypothetically yielded a nonzero TE, this particular 30min trial's result can be attributed to variation arising from lack of consistency in protocol, which is elaborated upon in the Conclusion section. Thus, greater lengths of multimeric DNA are more efficient for transformation in *Bacillus subtilis.*

The smearing in the 30min RCA colony lane and absence of bands in the O/N RCA colony lane indicate that the lysozyme was likely not given enough time to fully break down the bacteria's cell walls. Still, the presence of the colonies signifies that they have chloramphenicol resistance, a feature arising from a gene found on the plasmid, which suggests that the colonies were successfully transformed.

ACKNOWLEDGEMENTS / REFERENCES

Special thanks to Angela Merchant for allowing us to use her lab, and Anming Xiong, who came into our lab every week to guide and supervise us. They both poured in an incredible amount of time, effort, resources, and energy to guide and inspire us during this project.

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