Engineering a Butyrate Pathway in *E. coli Nissle 1917*

William Owens,[1] Max Van Belkum[1], Pedro Corral[2], Ameer Basta[2], K. T. Shanmugam[1], Christopher Reisch[1]

[1]Department of Microbiology, [2]Department of Chemistry, University of Florida, Gainesville, FL

Introduction

Butyrate is a short-chain fatty acid (SFCA) that promotes apoptosis in colon cancer cells, regulates inflammation, and has many other probiotic benefits. Many researchers have previously engineered butyrate pathways in *E. coli*. One student team from the University of Toulouse produced an *E. coli* pathway, but did reported no butyrate synthesis[2]. Probiotic *E. coli* strain *Nissle 1917* can colonize the human gut[3], and has been previously engineered for therapeutic purposes[4]. With a proper butyrate pathway, *Nissle* would be a potent probiotic and therapeutic agent.

Hypothesis

We hypothesized that *Nissle*—when endowed with a pathway containing genes *atoB*, *hbD*, *crt*, *tesB*, and *ter*—would produce copious quantities of butyrate during anaerobic fermentation. Furthermore, we hypothesized that the Toulouse pathway failed due to poor redox balance and would function correctly with *ter* added.

Methodology

Primary Approach:

- Order our designed operon from IDT in two fragments
- Join the two fragments together using either traditional restriction enzyme assembly or overlap extension PCR[4]
- Transform both *Nissle* mutant and reference strain (BEM3) with constructed pathway
- Screen for successful transformants and confirm via PCR
- Using HPLC, compare fermentation products of each strain with and without the engineered pathway

Secondary Approach:

- Transform *Nissle* and *BEM3* with the Toulouse pathway
- Screen for successful transformants and confirm via PCR
- Transform the successes from previous step with *ter* in plasmid pSB1C3
- Screen for successful transformants and confirm via PCR
- Using HPLC, compare fermentation products of each strain without any added parts, with only the Toulouse pathway, and with the Toulouse pathway and *ter*

Results

The designed pathway could not be assembled with overlap extension or restriction enzyme digestion followed by ligation. We suspect that our restriction sites and homologous regions were disrupted after the supplier optimized the fragments. As a result, we reverted to our secondary approach.

The secondary approach proved more fruitful. Both the *Nissle* mutant and the *BEM3* mutant were transformed with both Toulouse pathway. These transformants were then transformed with the *ter* part. (Note that this *Nissle* mutant had two competing genes, *ldhA* and *frdA*, deleted. The *BEM3* mutant was provided by the Shanmugam lab, and all of its competing genes were eliminated.)

In Nissle (Figure 1), we observed marginal butyrate production in all 3 samples, casting doubt on this trial's validity. In *BEM3* (Figure 2), we observed a significant adjusted butyrate production in only the sample endowed with both the Toulouse pathway and the *ter* part.

Figure 1
Butyrate Production in Nissle

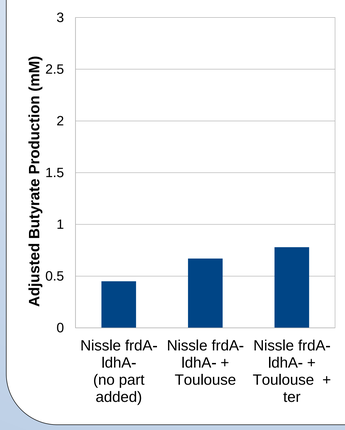
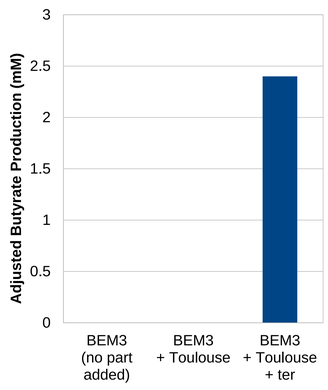


Figure 2
Butyrate Production in BEM3



Conclusions

- Our failure to synthesize the original pathway highlights the importance of planning redundant strategies in synthetic biology.
- HPLC data for *Nissle + Toulouse + ter* is questionable. Our *Nissle 1917* mutant likely contains competing genes that disrupt redox balance.
- HPLC data for the redox-balanced *BEM3* mutant strongly suggests that the Toulouse pathway functions when supplemented with the NADH-dependent *ter.*

Future Work

With additional time and resources, further gene deletions could make our *Nissle 1917* mutant better suited for butyrate synthesis, and our pathway could be modelled and optimized further. Our novel Toulouse + *ter* system has been made publicly available on the BioBrick repository for other researchers to innovate upon.

References

1.Canani et al. "Potential Beneficial Effects of Butyrate in Intestinal and Extraintestinal Diseases." World Journal of Gastroenterology : WJG 17, no. 12 (March 28, 2011): 1519–28. doi:10.3748/wjg.v17.i12.1519.

2. http://2015.igem.org/Team:Toulouse/

3. Scaldaferri et al. "Role and Mechanisms of Action of Escherichia Coli Nissle 1917 in the Maintenance of Remission in Ulcerative Colitis Patients: An Update." World Journal of Gastroenterology 22, no. 24 (June 28, 2016): 5505–11. doi: 10.3748/wjg.v22.i24.5505.

4. Bryksin et al. "Overlap Extension PCR Cloning: A Simple and Reliable Way to Create Recombinant Plasmids." BioTechniques 48, no. 6 (June 2010): 463–65. doi: 10.2144/000113418.

Acknowledgements

Thank you to the University Scholars Program for sponsoring this project, to my fellow team members for all their hard work, and most importantly to Dr. Christopher Reisch and Dr. K. T. Shanmugam, whose lab spaces and advising made this project a reality.