Investigating the role of regulation in resilience of microbial communities under fluctuating environmental conditions.

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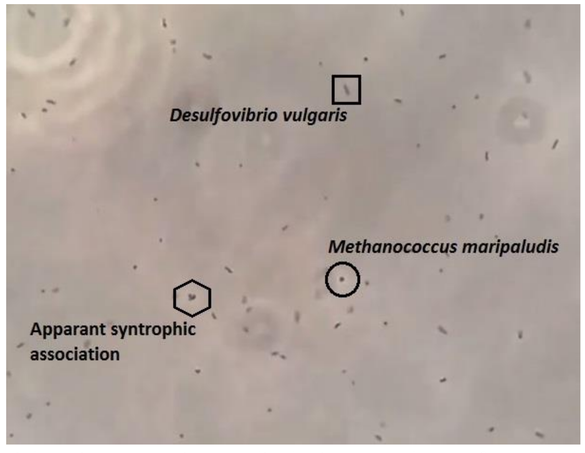
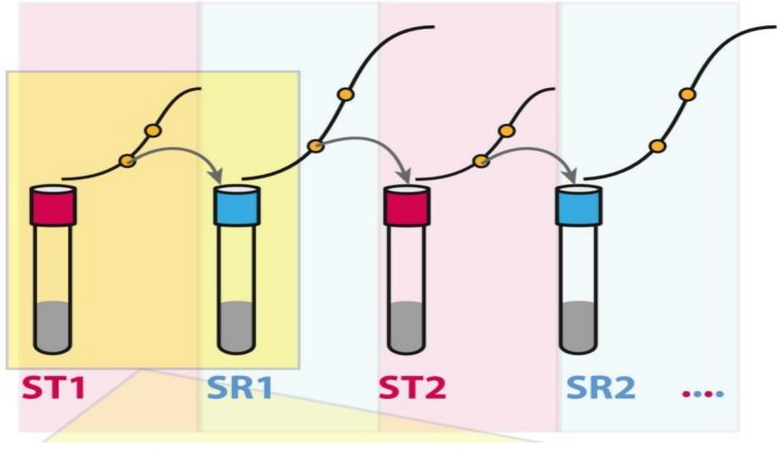


Light microscope image displaying co-culture of DvH and Mm (100x resolution).



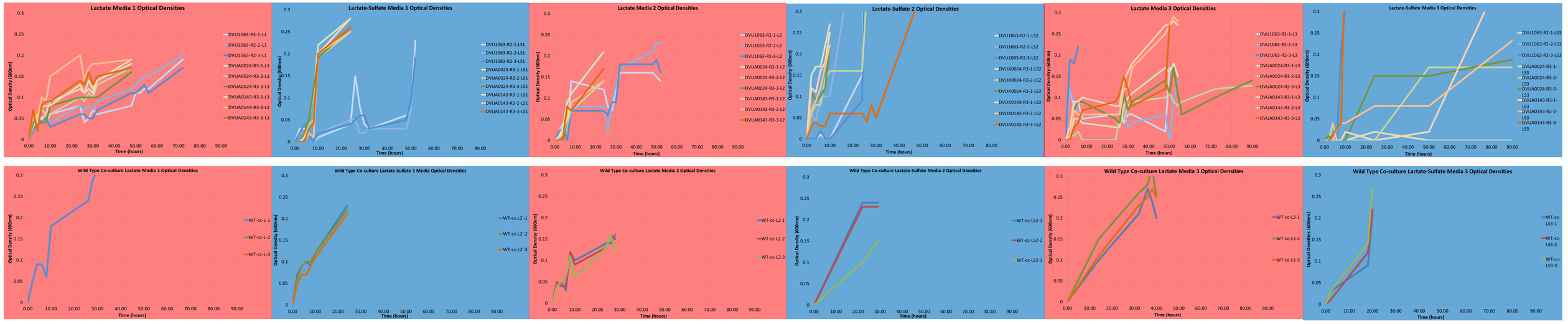
Depiction of transition experiments workflow (Turkarslan 2017).

Methods

- *Desulfovibrio vulgaris* Hildenborough (DvH) transcription factor (TF) mutants were co-cultured with wildtype *Methanococcus maripaludis* (Mm).
- Cultures were incubated at 37˚C in anaerobic Balch tubes with at pH 7.2.
- Lactate media was used to induce syntrophic growth and lactate-sulfate media was used for sulfate-reducing growth by DvH.
- Growth was measured with a spectrophotometer (at 600nm).
- Anaerobic transition experiments began in lactate media. At OD ~0.15, 0.5ml inoculum was transferred into new lactate-sulfate media. At OD ~0.20 in lactate-sulfate media, 0.5ml inoculum was transferred into new lactate media.
- RNA was sampled at transitions 1, 4, and 8 by centrifuging, then flash freezing the pellet with liquid nitrogen. MasturPure Complete kits were used to isolate RNA prior to sequencing with Agilent RNA 6000 Nano kits.

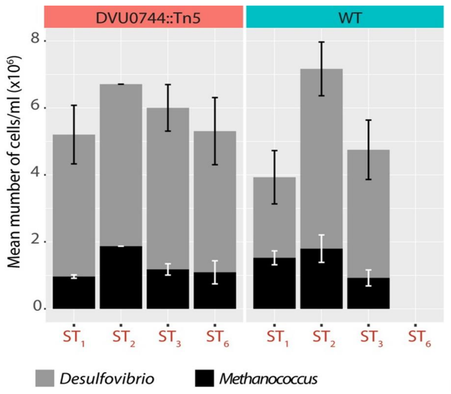
Mentorship & Collaboration

As a part of my internship, I volunteered to mentor high school students teaming up with me to conduct a research project. Since I entered the project the same time as them, we were learning the protocols **simultaneously**. However, I took note that **Georgia and Lauren** needed guidance in interpreting research articles and database information to benefit their project. I helped them seek out and extract, and summarize useful and relative information. For our project, we had to find information on genes, proteins, and their interactions pertaining to our experiments. I presented interactive databases to them, such as STRING, to aid their background knowledge on their mutant strains of *Desulfovibrio vulgaris*. After I assisted them with their research, **they were able to conduct their projects on their own.** Eventually, they exceeded expectations and were designing assistive tools to make the experimental process more efficient. For example, they initiated an outline for a spreadsheet to input optical density data and graph growth patterns. **They were crucial in recording time points and producing growth media.** They successfully worked on their experiments together, thinking collectively to solve issues with minimal assistance.

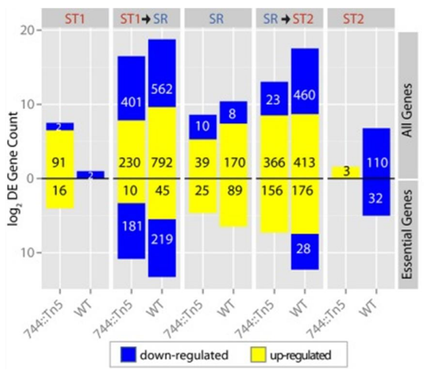


Discussion

Despite the unusual growth patterns of co-cultures, there was a general increase in growth. Mutant DVU1063 grew at a much slower rate than mutants DVUA0024 and DVUA0143. Possibly, DVU1063 is an important gene but not essential since there was growth. Microbesonline, STRING, and Syntrophy Portal show DVU1063 is associated with upregulation of sigma-54 dependent transcription factors. If the gene is knocked out, it is likely important transcription factors are not being expressed. The knockout of DVUA0024, a sigma-54 interaction domain protein, slightly impeded growth. Its general function is vague but there is indication it regulates RNA polymerase and transcription factor binding to DNA. DVUA0143, a nif-specific regulatory protein, was the fastest growing. Nif-regulatory genes are involved in nitrogen fixation and the headspaces of the cultures are filled with nitrogen. But the given conditions and gene knockout still resulted in rapid growth for this mutant. None of the cultures completely collapsed. It was expected for DVU1063 to collapse early, but it could have been merely growing at a slow rate. After RNA sequencing data is obtained, the expression levels of genes will give insight on the essentiality of relevant genes. The RNA sequence data supplemented by databases will help elucidate regulatory gene networks of *Desulfovibrio vulgaris*.



Turkarslan (2017) shows that collapse is not due to "dilution" of Mm in co-culture.



Previous experiments show collapse can be due to downregulation of essential genes due to knockout via transposon mutants (Turkarslan 2017).

References

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