A High-Throughput Survival Assay to Assess the Molecular Mechanism of Desiccation Tolerance in Tardigrades

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Tardigrades

Tardigrades are microscopic animals that survive:

- -272 °C to 151 °C[1-2]
- 10 days in space[3]
- 10+ years dehydrated[4]
- 30+ years frozen[5]

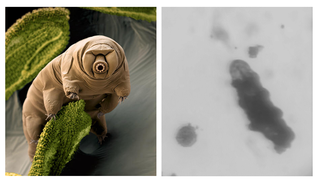


Figure 1. Tardigrades

Left source: Eye of Science

Cytosolic-abundant heat-soluble (CAHS) proteins [6]

- Unique to tardigrades
- Necessary for desiccation tolerance[7]
- Enhance desiccation tolerance of other organisms[7]

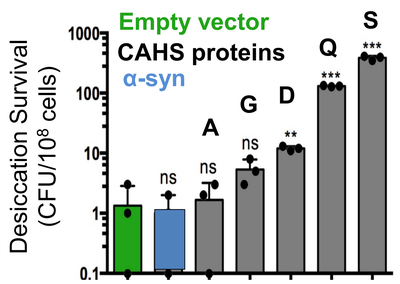


Figure 2. Desiccation survival of *E. coli* expressing different proteins. Adapted from (7)

Goal: Develop a high-throughput survival assay to determine which amino acids in CAHS proteins are important for desiccation survival.

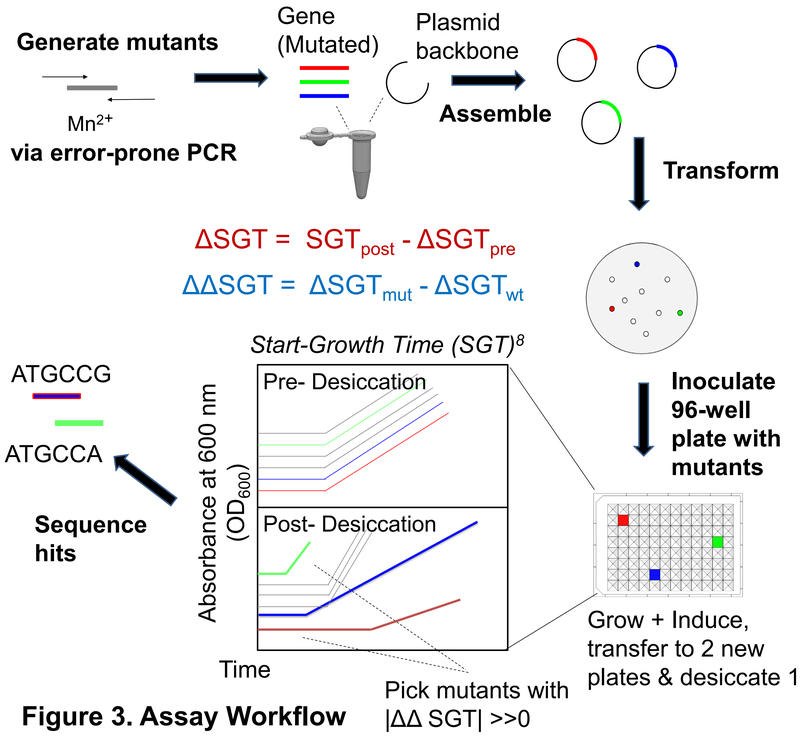


Figure 3. Assay Workflow

Calibration

Table 1. Colony Forming Units (CFU) vs. OD_{600}

Trial	OD	CFU/mL	Avg. CFU/mL (OD 1)	Standard deviation (P)
1	1.669	1.52E+08	1.19E+08	2.76E+07
2	1.606	2.35E+08		
1	1.618	1.91E+09	1.20E+09	1.97E+07
2	1.828	2.23E+09		

Induced Not induced

Figure 4. Calibration of plate reader to spectrophotometer

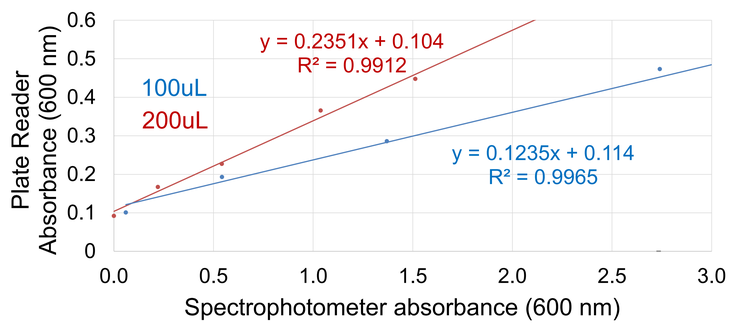


Figure 5. Growth curves of serially-diluted *E. coli*

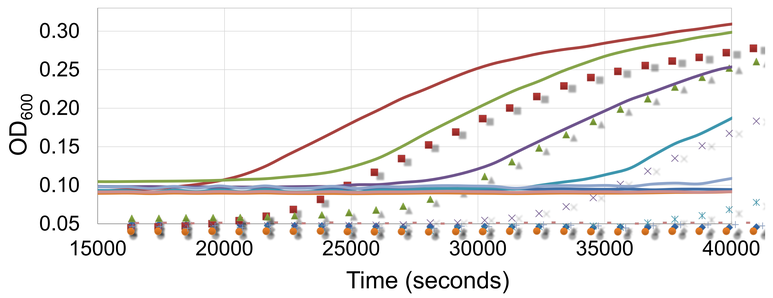
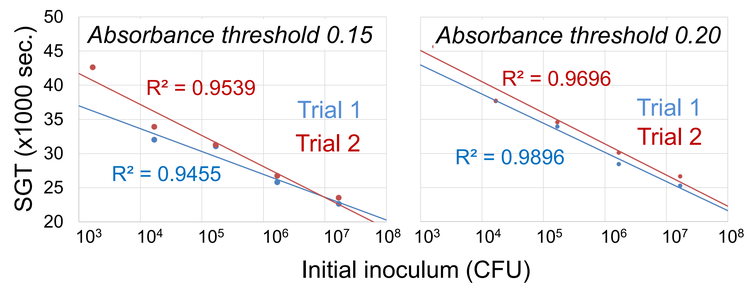


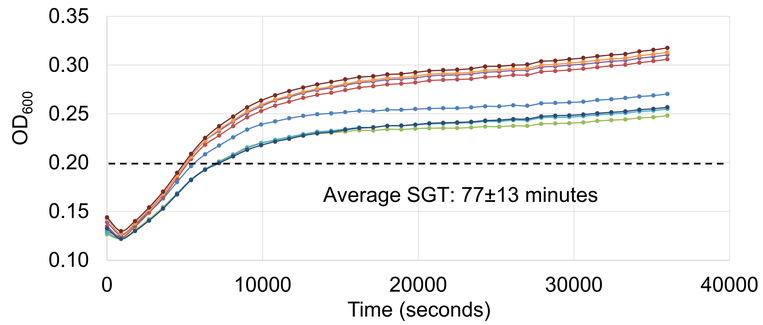
Figure 6. SGT vs. cell density of initial inoculum



Points represent average SGTs of serial 1/10 dilutions in a 96-well plate, n =12 per point. SGT defined by the time when cultures reached an absorbance of 0.15 (left) and 0.2 (right). $\Delta SGT_{1/10}$ = 54.3 for an absorbance threshold 0.15 and 68.9 minutes for a threshold of 0.2.

Variability

Figure 7. Row-averaged OD_{600} vs. time (96-well plate)



E. coli expressing CAHS G from a single day culture were diluted 1/20th and grown at 37ºC overnight in a 96-well plate. Absorbance was read every 15 minutes over a 10 hour period. SGT was defined as the time at which OD_{600} reaches 0.2.

Table 2. Overnight cell culture volume and density

Volume	Average Absorbance (600nm)	CV (%)
1000 uL	0.2	20
500 uL*	0.44	35
200 uL	0.64	16

Future Directions

- Determine [Mn^{2+}] for an error rate of five mutations/kb
- Optimize homogeneity
  - Overnight growth volume and time
  - Desiccation method
- Run assay on different protein controls and random mutants of CAHS proteins

Our assay will help us understand how CAHS proteins protect against desiccation, which may lead to more effective methods for stabilizing protein-based drugs.

References and Acknowledgements

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