Different RsbR Paralogs in *Bacillus subtilis* Affect Cell Viability When Exposed to Environmental Stress

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Abstract

Stress is a universal phenomenon experienced by all living organisms. Bacteria have to react to stress quickly in order to survive in their environment. Environmental stress can be caused by a variety of factors, including acid, alcohol, salt, and heat. We are studying the model bacterium *Bacillus subtilis*, because its stress response resembles that of human pathogens such as *Listeria*. *B. subtilis* senses stress using a stressosome, a complex of 80 proteins that includes four variant RsbR protein paralogs, each of which produce a distinct stress response pattern to an identical stressor. We know that these four RsbR proteins work together to aid in survival in the presence of environmental stress in wild-type cells (WT), which contain all four RsbR proteins; however, we do not know how each RsbR protein affects cell fitness. To test how each individual RsbR protein affects survival, we performed a competition assay pairing strains containing individual RsbR variants against each other to determine if one protein aided in cell survival more than the other. To do this, we engineered strains of *B. subtilis* cells to only contain one of the four RsbR proteins. This allowed us to compete the RsbR proteins against one another on an individual basis. Our preliminary results indicate that the wild-type strain substantially outcompeted the other strains in every competition assay performed under acid stress except when competed against RC. These results show that cells containing only RC or all four RsbR proteins have a higher fitness in acid stress than cells containing only RA, RB, or RD.
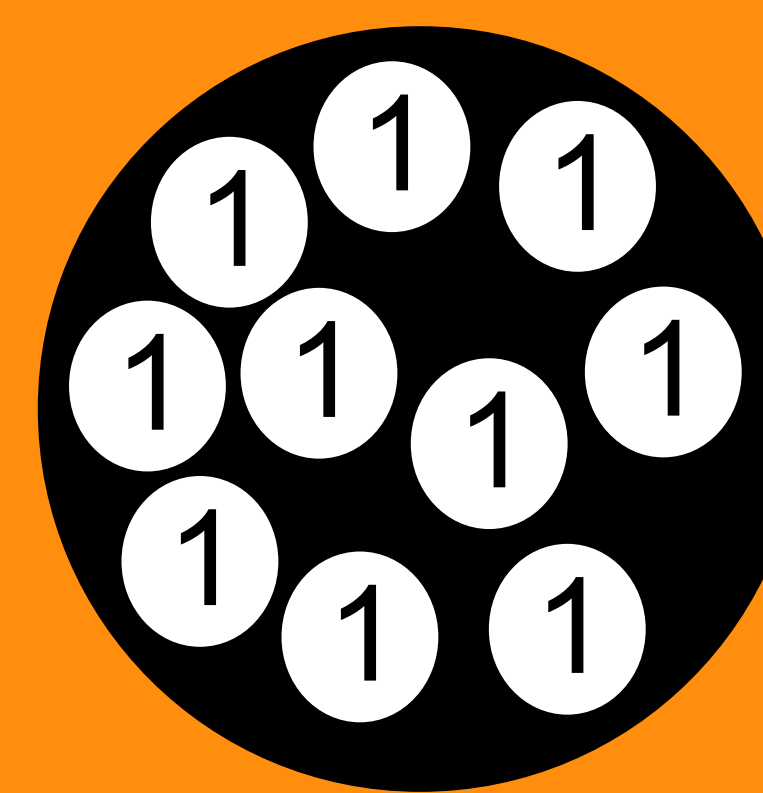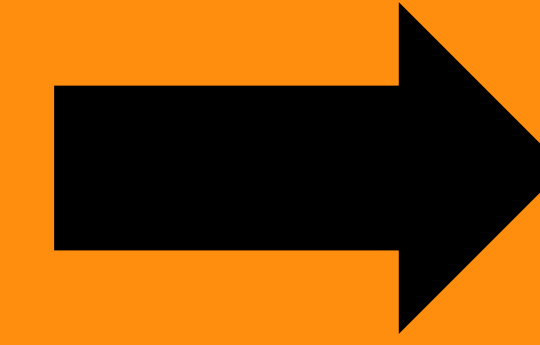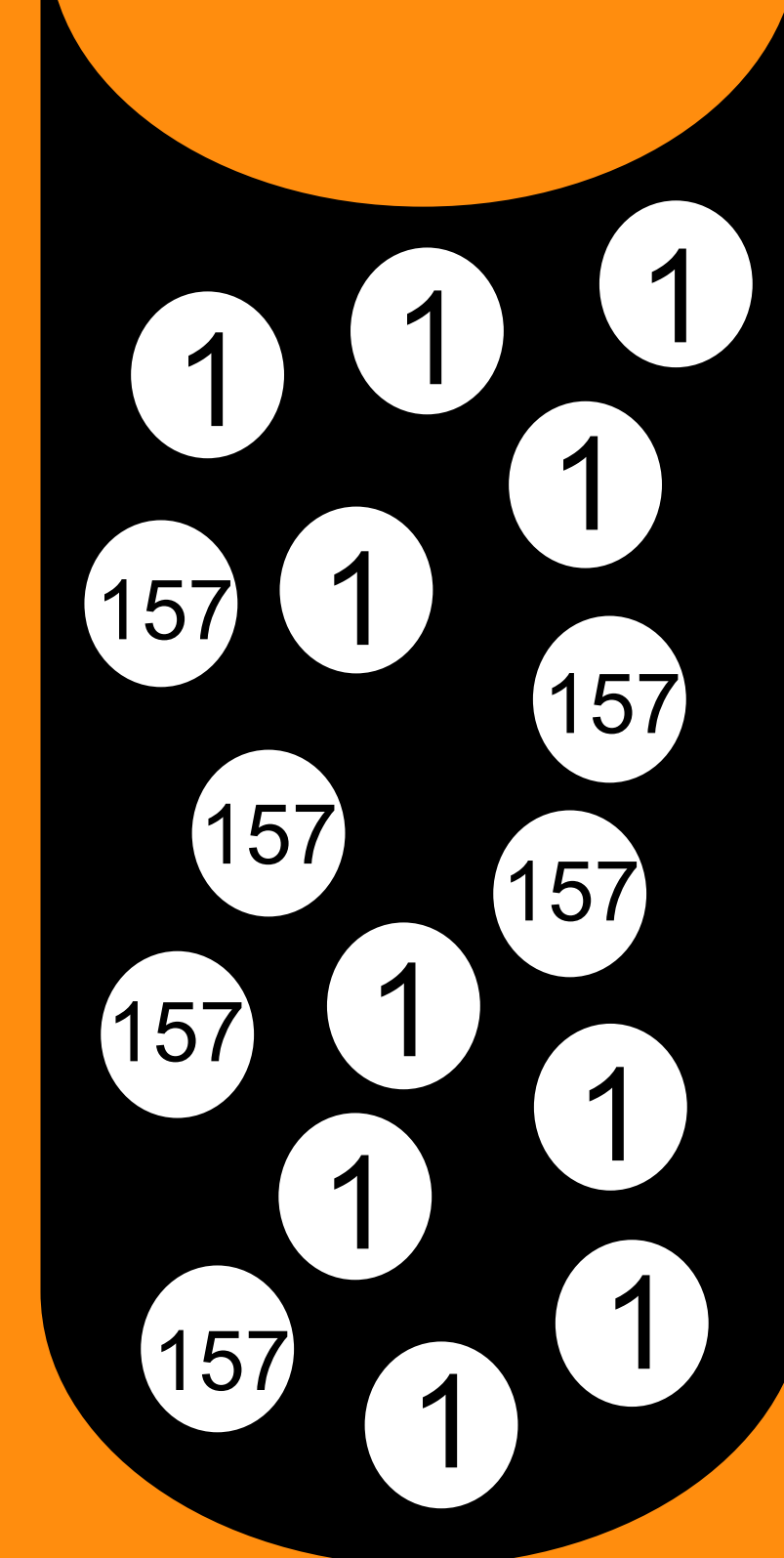
Hypothesis

We hypothesize that strain marked only with RsbRC will outcompete all of the other strains, including the WT strain, in the presence of other environmental stressors.

Methods

We competed pairwise combinations of the strains in identical environmental stress. We inoculated equal amounts both strains in a flask starting at a pH of 6.5, then introduced a pre-determined volume of HCl (at timepoint T=0) to lower the pH of the culture to 6.25. This was necessary for the cells to experience a difference in pH (acid stress). Every three hours, we diluted the strains into a new flask of pH=6.25 to keep the culture in exponential phase and keep the cells under continuous stress. We also plated a sample of the culture every three hours onto six agar plates, three of which were marked with Kanamycin resistance and three of which were marked with Chloromycin resistance. Each strain was also pre-labeled with either Kan or Chlor so that only one of the strains will grow on each plate. After plating, we counted the colonies produced on each plate to compare the fitness level of each strain, which is shown in **Figure 3.** This assay is visualized in **Figure 2.**

FIGURE 1

KEY
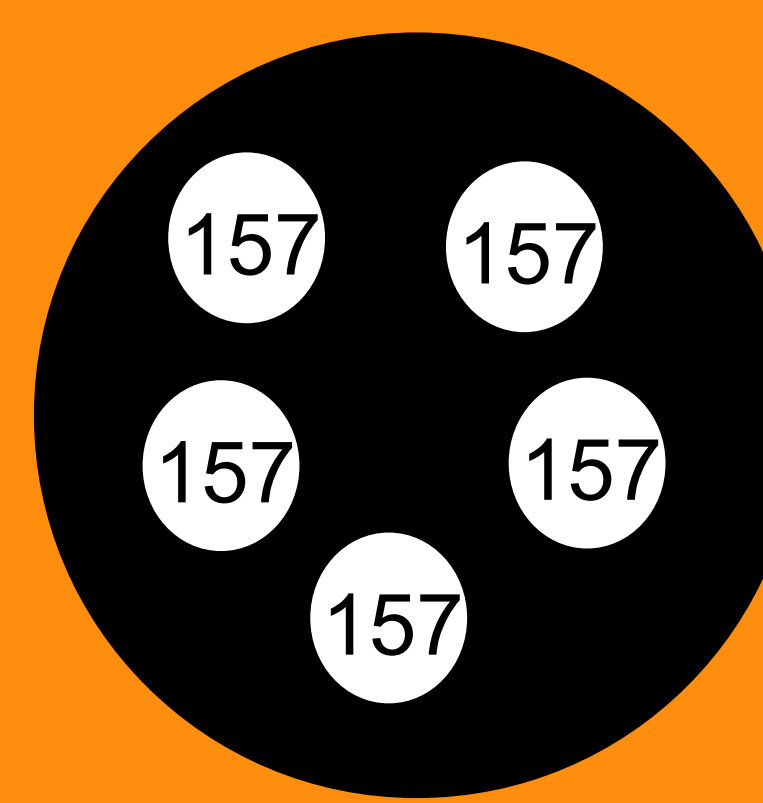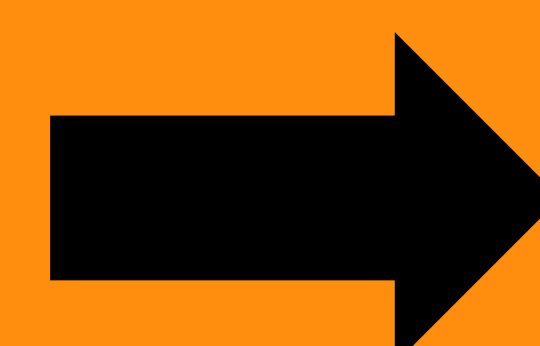
Strain 1 (WT w/KAN resistance)
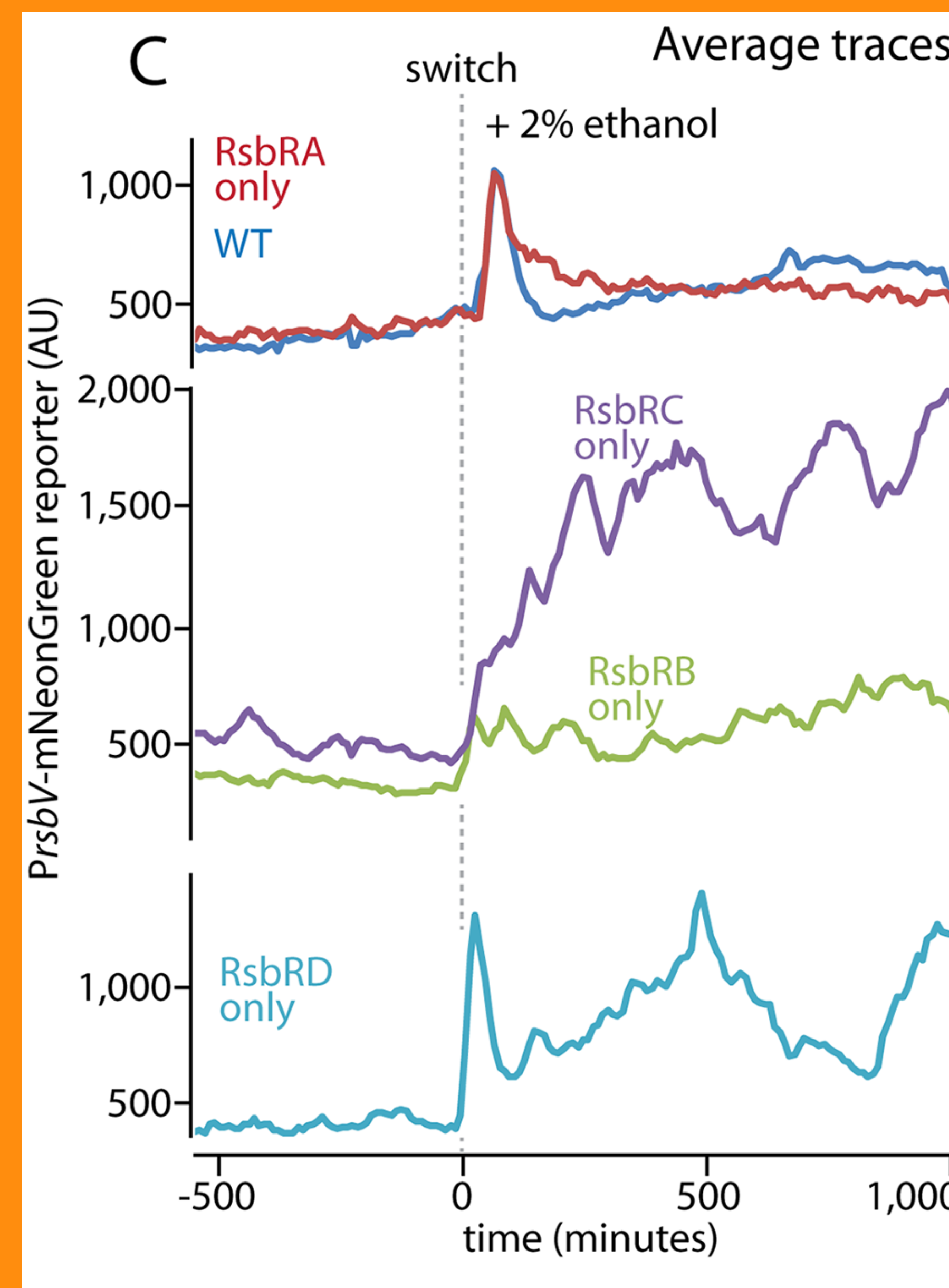
Strain 157 (RsbRA w/CHLOR resistance)

FIGURE 2. Each RsbR protein exhibits a unique stress response pattern

FIGURE 3

Results

Our hypothesis has held true with the data obtained thus far. It has become clear to us that the RsbRC paralog is extremely advantageous to the cell during stressful conditions. This is likely due to the ability of RsbRC to stay activated for extended periods of time as opposed to oscillations of activation in the sister paralogs of RsbRC.

References

Cabeen MT, Russell JR, Paulsson J, Losick R. (2017) Use of a microfluidic platform to uncover basic features of energy and environmental stress responses in individual cells of *Bacillus subtilis*. *PLoS Genet* **13**: e1006901.