# Stress Response of RsbR Protein Paralogs in *Bacillus subtilis* Under 1M Sodium Stress

**Sidney Bush, Madeline Toews, Matthew T. Cabeen**

## Introduction

Stress is a universal phenomenon, and so all organisms must employ a way to cope with common stressors. (Hazan et al. 2004). Bacterial stress is limited to environmental factors that make it difficult for the organism to either survive or reproduce. For this reason bacteria need a mechanism in which to deal with these conditions. Within model organism *Bacillus subtilis*, a gram-positive, and noninfectious bacterium, resides a complex of proteins known as a stressosome. The stressosome is responsible for sensing common environmental stressors such as ethanol, sodium, and pH changes, and signaling a cascade of genes to turn on in order to promote a general stress response. In each cell, there are about 20 individual stressosomes.

Nearly all bacteria with stressosomes rely on gene regulation for an effective stress response. The protein that reacts to stress in *B. subtilis,* labeled $\sigma^B$, is inactive because of the anti-sigma factor (RsbW) binding to it and not allowing $\sigma^B$ to bind with RNA polymerase to initiate a stress response. When stress is present in the environment, The RsbR protein, what we believe to be responsible for sensing stressors in the environment, initiates a series of events leading to the dephosphorization of the anti-anti-sigma factor (RsbV) so that it may bind with bind with RsbW. This causes RsbW which was previously bound to $\sigma^B$ to release it. (Benson et al. 1993). From here $\sigma^B$ is then free to bind with the RNA polymerase, initiatinga a cascade of

genes to promote a general stress response throughout the cell (Feklístov et al.)

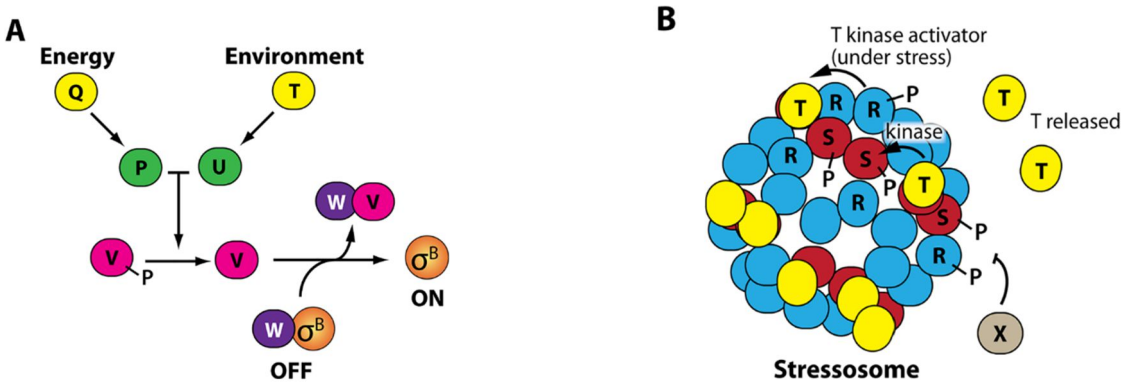


Figure 1: (A) The signaling pathway in which environmental stressors are sensed and later promote a stress response. (B) Diagram of what a stressosome conceptually looks like and how stress (x) is sensed.

Within the stressosome of *Bacillus subtilis* are four different paralogs, or variants, of the RsbR protein: RsbRA (RA), RsbRB (RB), RsbRC (RC), and RsbRD (RD). In a WT *B. subtilis* bacterium, the stressosome contains all four paralogs. We know engineered strains possessing only one of the four paralogs, respond to an identical ethanol stress differently (Cabeen et al. 2017). We do not know how these engineered cells react to the common stressor sodium chloride (NaCl), and which variant is best at helping the cell to survive during exponential phase. We predicted that the wild type strain would display the best overall response as it has all four of the paralogs present.

## Methods

We first chose the strains of bacteria to be competed. One of these strains must be resistant to the antibiotic chloramphenicol (chlor) and the other must have a kanamycin (kan) resistance so that we could later separate and distinguish the strains. We plated these strains on separate Lennox broth plus agar (LB) plates with the appropriate antibiotics, for the growth of each strain. We allowed the plated strains to grow for 24 hours. We then inoculated a tube of 3

ml of potassium buffered Lennox Broth (LBK) medium with one plated strain and repeated with the other strain in a separate tube. We placed these into the shake incubator and allowed each to grow overnight. We diluted each culture the next day 100x into 25 ml LBK so only actively growing strains are being competed. We waited until the spectrophotometer measured an absorbance of the diluted strains at $OD_{600}$ that was above 0.1 (typically about an hour), and then diluted each strain, to have 12.5 ml of each strain at an absorbance of 0.1. We then combined the two strains together in sterile 1M NaCl LBK to make a solution of 25 ml 1M NaCl LBKwith an absorbance of 0.1 that is equal parts each strain. We placed the solution into a shake incubator for about a minute. We took a 1 ml sample of this solution as our 0min. (T0) timepoint, and returned the flask back to the shake incubator. We took the absorbance of 500 µl of the T0 sample and conducted a serial dilution with what remained. (100 µl sample into 900 µl sterile LBK for a $10^{-1}$ dilution; take 100 µl of the $10^{-1}$ dilution and place into 900 µl sterile LBK for a $10^{-2}$ dilution. Repeat until at a $10^{-7}$ dilution). We plated 100 µl of each dilution on both Chlor plates, and Kan plates to distinguish the competing strains. Before the solution reached an absorbance of $OD_{600}$ .5 we diluted back to $OD_{600}$ .1 to remain in exponential phase. Our team repeated these steps at 3 hours (T3), 6 hours (T6), and 9 hours (T9), diluting as nessisary. We allowed the plates to grow for 24 hours and counted colonies. We determined which strain out competed the other by comparing the colony counts and determining percent of total population. As control experiments, we competed nearly identical strains with different antibiotic markers, (wild type vs. wild type, strain A vs. strain A etc.) to make sure the antibiotic resistance did not interfere with our results.

## Results:

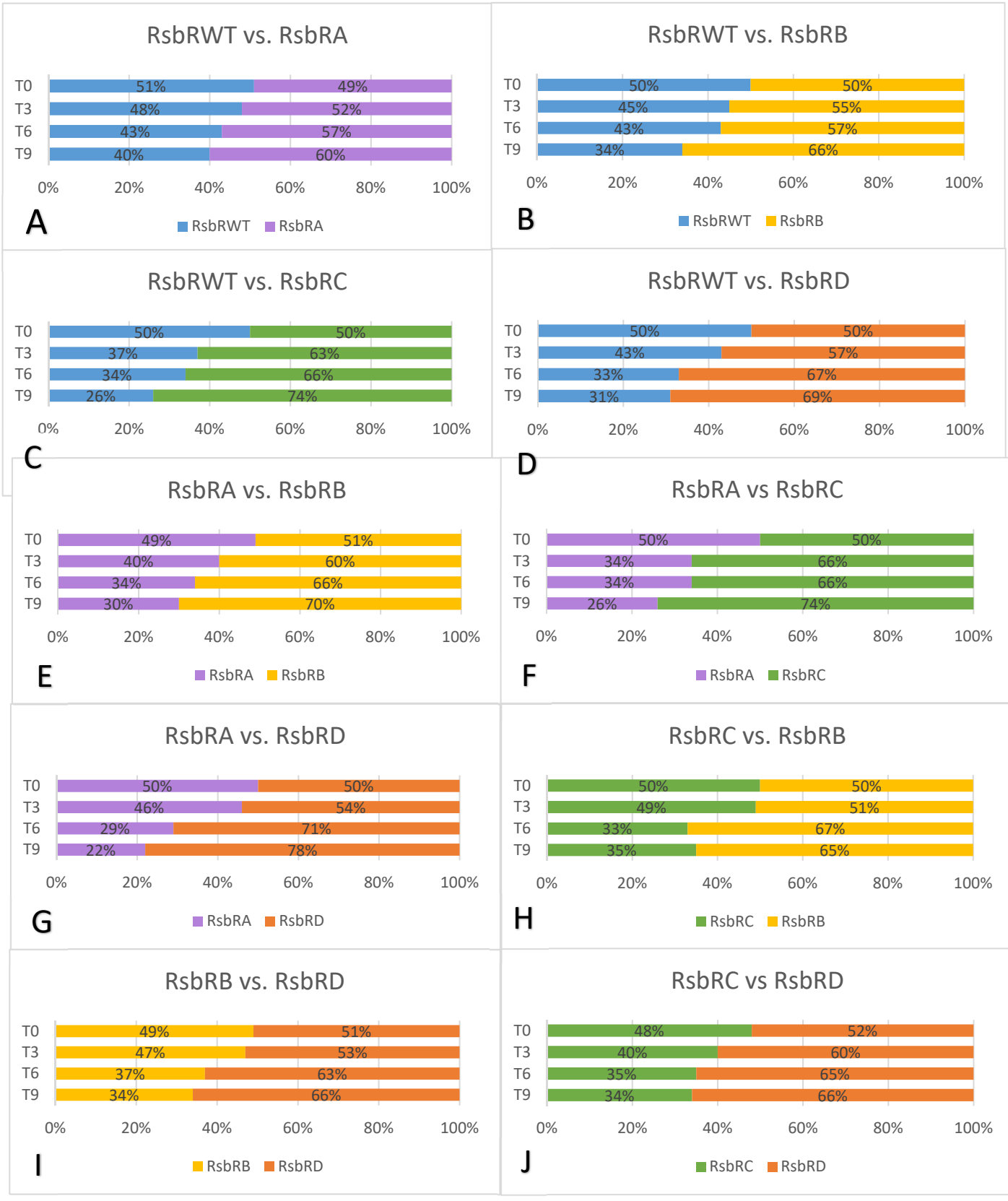


Figure 2: Percent of total culture in competition assays at 180, 360, 540, minutes. Part A shows the competition of WT and RA strains. Part B shows the competition of WT and RB strains. Part C shows the competition of WT and RC strains. Part D shows the competition of WT and RD strains. Part E shows the competition of RA and RB strains. Part F shows the competition of RA and RC strains. Part G shows the competition of RA and RD strains. Part H shows the competition of RB and RC strains. Part I shows the competition of RB and RD strains. Finally, Part J shows the competition of RC and RD strains.

All tests were performed in exponential growth (without stress absorbance is about doubling every 20 minutes). We determined percent values through colony counts at the same dilution. Figure 2 shows the change in percent concentration of each competed strain over time. All experiments took place over the span of 2 semesters (12 months).

## Discussion

Our data does not support our hypothesis of the wild type promoting the best overall survival. When all strains were competed under identical 1M NaCl stress we saw, in exponential phase, the order of out-competition being: RD being most fit, followed by RB, RC, RA, and lastly, wild type (Figure 2). All this considered, in terms of strongest overall response under these conditions we can conclude RD promotes the greatest overall fitness, followed by RB, RC, RA, and finally the wild type. This means that the RsbR paralogs are likely optimized for different specific stressors as opposed to our previous hypothesis of wild type being the most fit under all different stressors. A possible confounding factor in our results was that if there was excess moisture on the plate. Under this condition, multiple colonies could appear as one large one leading to an underestimated cell count. We avoided this to the best of our ability by placing the plates in a biological safety cabinet for 30 minutes to dry before plating our timepoints. Another possible error could have been inaccurate readings on the spectrophotometer. If we were to accidentally put unequal parts into each flask we would see one strain falsely showing out-competition due to greater numbers at the beginning.

In future experiments we will continue competitions in different common stressors such as Ph, oxidation, and antibiotics. to compare results of that experiment to our findings in this one. From such experiments we could determine if different paralogs demonstrate higher fitness in

the presence of different stressors or if the RD paralog promotes the greatest fitness across multiple stressors. Also we would like to explore how the stressosome is able to sense these stressors. The stressosome being inside the cell would imply it cannot access the outside of the cell to test for these stressors.

Finally, the importance of these experiments are to investigate the ways in which bacteria are able to respond to stress. The stressosome is not entirely unique to *B. subtilis,* other common pathegens such as *Bacillus cerus* and organisms in the genus *Listeria* have similar mechanisms by which they respond to stress. By learning more about this mechanism we may be able to find new and better ways to combat infections in stressful environments such as the human body.

## Literature cited


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