Influence of a Guanidine Riboswitch in Bacterial Cells

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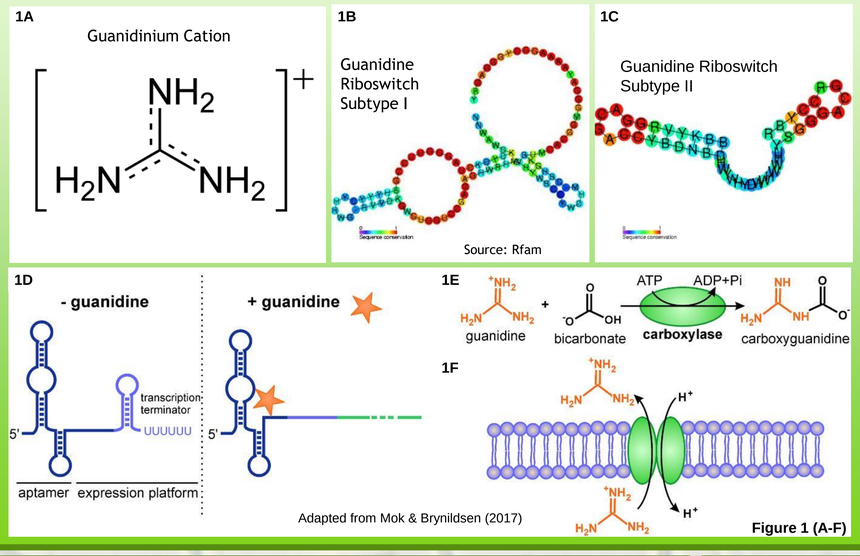
Introduction

We measured the effect of guanidine (Fig. 1A) on bacterial growth, and investigated guanidine riboswitches, subtypes I and II (1B, C). One of many ways bacteria regulate protein expression, riboswitches are segments of mRNA containing two regions: the aptamer and the expression platform. The aptamer binds a small molecule, causing a conformation change in the expression platform, activating translation (1D). Guanidine is often toxic to bacterial cells, and guanidine riboswitches regulate the expression of proteins that make it less toxic (1E) or export it out of the cell (1F).



Bacterial Growth & Guanidine Toxicity

Two strains of *Pseudomonas* and five bacterial strains that interact with algae were measured for growth (Fig 3A-B). Growth data were fit to the model equation (Fig. 4A) to derive growth rates and maximum population yields (4B-D). Note that although *Pseudomonas* strains have the highest yields, algal-associated species such as *Marinobacter* sp. PT19DW did not slow in growth rate until the highest level of guanidine (100 mM), while growth of *Pseudomonas protegens* Pf-5 declined in 10 mM guanidine.

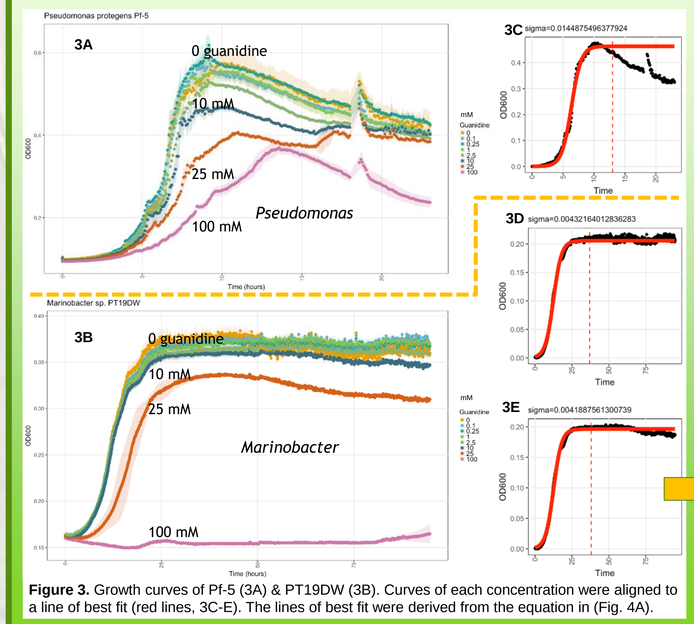


Figure 3. Growth curves of Pf-5 (3A) & PT19DW (3B). Curves of each concentration were aligned to a line of best fit (red lines, 3C-E). The lines of best fit were derived from the equation in (Fig. 4A).

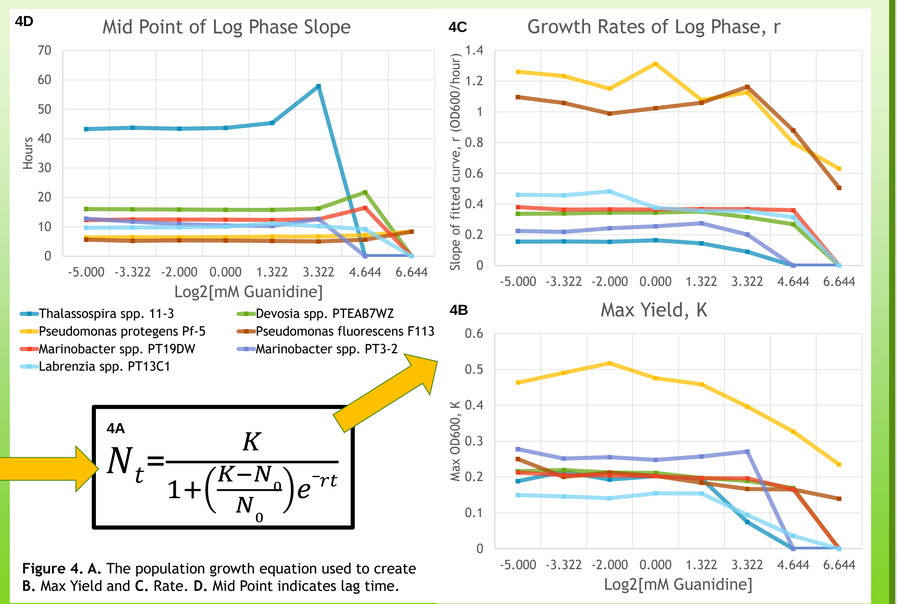


Figure 4. A. The population growth equation used to create **B.** Max Yield and **C.** Rate. **D.** Mid Point indicates lag time.

Sequence & Structure of Guanidine Riboswitches

Analysis of genome sequences indicate the bacteria we studied contain riboswitches with structural similarity to confirmed guanidine riboswitches (Fig. 2). Therefore, we tested these bacteria for tolerance to guanidine.

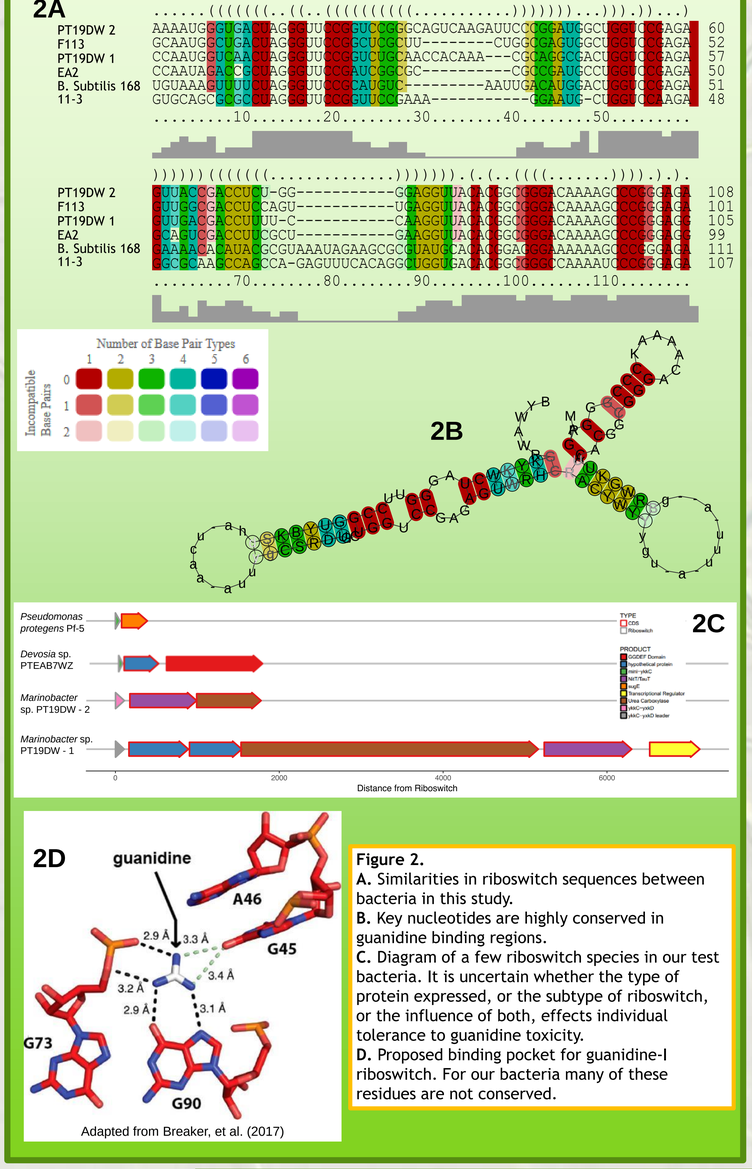


Figure 2.
A. Similarities in riboswitch sequences between bacteria in this study.
B. Key nucleotides are highly conserved in guanidine binding regions.
C. Diagram of a few riboswitch species in our test bacteria. It is uncertain whether the type of protein expressed, or the subtype of riboswitch, or the influence of both, effects individual tolerance to guanidine toxicity.
D. Proposed binding pocket for guanidine-I riboswitch. For our bacteria many of these residues are not conserved.

Cyanobacterial Response to Guanidine

We measured the growth of the model cyanobacterium ESFC-1 (Elkhorn Slough Filamentous Cyanobacteria-1) in the presence of guanidine (Figs. 5 and 6). Microscopy indicated that guanidine did not affect growth up to 10 mM (7A and 7C), but toxicity was apparent at 100 mM (7B and 7D). In wells containing 100 mM guanidine, the presence of N (as NO_3) in the medium (5B) may have stabilized the cells to some extent, and the cyanobacteria were less aggregated.

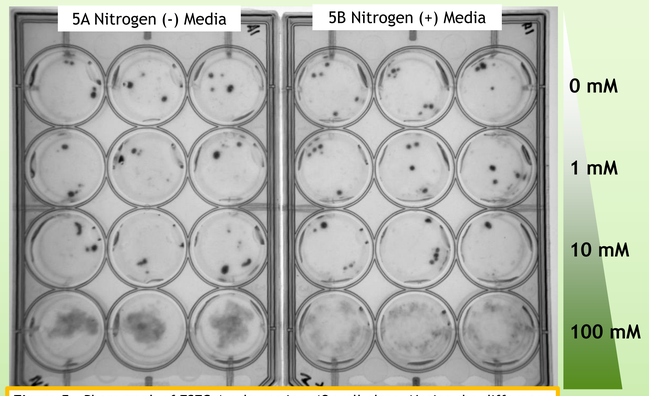


Figure 5 - Photograph of ESFC-1 cultures in a 12 well plate. Notice the difference in colony morphology at 100 mM.

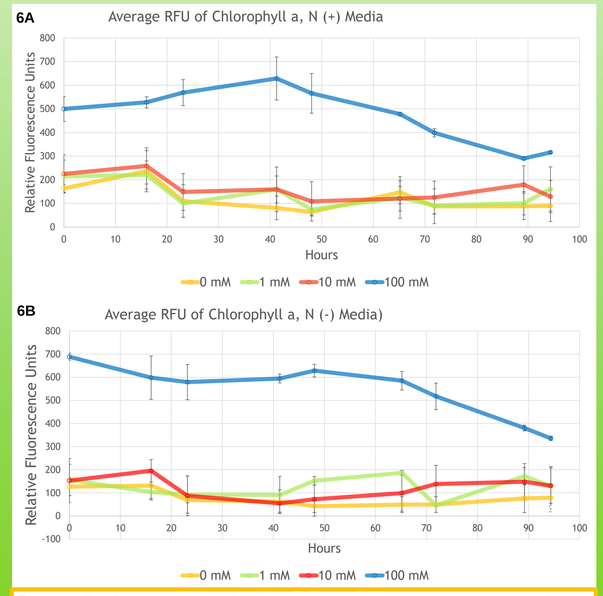
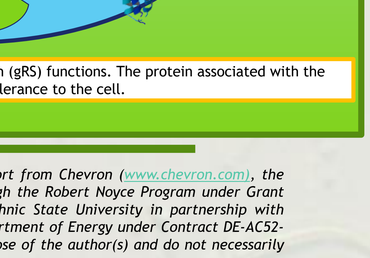


Figure 6. Relative Fluorescence Units (RFU) measurements for chlorophyll a in N-containing media (6A) and N-free media (6B). RFU measurements varied due to cell aggregation.

Figure 7 (A-D) - Brightfield images of ESFC-1 cultures at 20x magnification. Notice the difference between 10 mM and 100 mM treatments.

Methods

1. 12 and 96-well microplates were used with increasing concentrations of guanidine, in a 10:1 ratio of bacterial culture to guanidine.
2. Plates were put in a plate reader and incubated for growth.
3. Growth was monitored for 24 - 120 hours: bacteria (O.D. 600nm), and cyanobacteria (460nm fluorescence).



96-well microplate set up scheme

Conclusions & Future Directions

- Within our overall project on improving algal productivity, the focus of this study is to: (a) describe various guanidine riboswitch sequences in bacteria that interact with biofuel producing algae; and (b) determine if guanidine has a positive or negative influence on the growth of the bacteria containing these riboswitches.
- The guanidine riboswitch may be a signaling mechanism between algae and their associated bacteria. Algae may provide guanidine to bacteria as a C source, and bacteria may provide a N source for the algae through the assimilation of guanidine. If bacteria are able to assimilate guanidine, they may be suitable partners for algal-bacterial interactions.
- ESFC-1 may use guanidine as a N source, but the results are inconclusive. 100 mM concentrations of guanidine are clearly toxic. At this level, guanidine may be disrupting biofilm formation through the possible denaturation of proteins involved in constructing biofilms.
- Genome sequence analysis of our guanidine riboswitches indicate that our test bacteria differ in four key highly conserved residues for a guanidine binding pocket (G45, A46, G73, G90). However, structures of the riboswitches may be highly similar, indicating their functions may be similar concerning guanidine binding as the ligand.

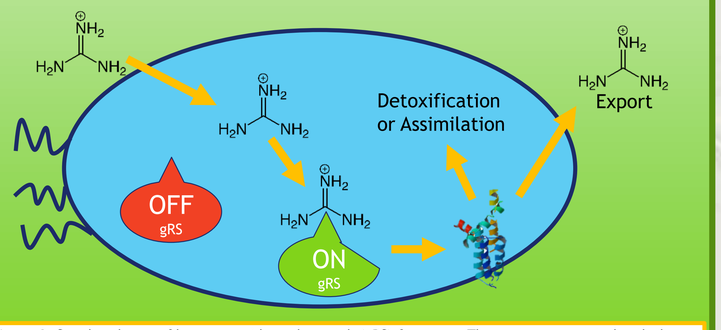


Figure 8. Simple scheme of how a guanidine riboswitch (gRS) functions. The protein associated with the gRS acts to modify or export guanidine and provides tolerance to the cell.

References

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