Simulation of an Engineered *Sinorhizobium fredii* Bacterial Expression System for Pesticide Removal in Soybean Plants

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Abstract:

Glyphosate is a heavily used herbicide commonly found in soybean plants that when consumed by humans, can cause long-term health complications from cancer, kidney failure, and reproductive issues. A novel bacterial expression system was designed to address glyphosate contamination in soybean plants and modeled using MATLAB Simbiology to observe glyphosate breakdown, resistance, and validation of a two-circuit mechanism. The proposed system utilizes a glyphosate-resistant strain of *Sinorhizobium fredii* that imports glyphosate from the environment into the cell and emits fluorescence and breaks it down afterward. The presence of these engineered bacteria in the root nodules can reduce glyphosate contamination in soy that is taken up and stored in the plant body.

Introduction:

Glyphosate (GP) is an herbicide used to kill a wide range of plants, disrupting the shikimic acid pathway through inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, inhibiting plant growth [1]. GP in the soil is absorbed by crops and after ingestion, can cause serious health risks such as human toxicity, eye or skin irritation, and nausea. GP may also threaten the life of birds and fish since it kills their food sources [1]. Research shows that Soybean plants contain nitrogen-fixing bacteria known as rhizobia. The synthetic biology mechanism described in this paper revolves around genetically modifying these bacteria to detect GP and break it down.

A strain of nitrogen-fixing bacteria known as *Sinorhizobium fredii* (SF) will be deployed as the host bacterial cell for this biological circuit. The bacteria is highly effective at breaking down soil nitrates and providing nourishment to plants [2]. It is unrivaled in its capability of modulating the roots of soybean plants and as a result of living within plant roots, these bacteria are exposed to the substances taken up by the plant first, acting as the first line of defense. Unlike other bacterial strains, SF also does not thrive in the soil and it lives optimally within nodules [3]. This is ideal for the goal of reducing GP within the plant, but not in the external soil allowing for GP to function still as a herbicide.

A summary of the circuit integrated into the host bacterial cell: In the absence of GP, the cell will synthesize *gltT*, a protein that actively transports GP into the cell from the external environment. In the absence of GP, the cell also synthesizes *glpA*, a molecule that provides GP resilience to the bacterial cell. Additionally, a regulatory molecule, *TrpR*, is also produced. *TrpR* represses a second part of the biological circuit which codes for more *glpA*, green fluorescent protein (*GFP* - to indicate the presence of GP in the cell), and *glpB* - a protein that breaks down GP inside the cells. Once GP has been completely degraded inside the cell, this second circuit is repressed and the initial circuit is reinitiated.

Materials and Methods:

The cloning of biobricks is first done to create enough available circuit parts. It will be performed using DH5α competent *E. Coli*, a versatile and commonly used host for cloning [16]. After part amplification, Next, purification, part digestion, and extraction will be used in preparation for the iGEM standard assembly. As a result of common antibiotic resistances among the different parts, standard assembly was a more desirable approach as opposed to the popular 3A assembly method (Table 1.)

Biobrick standard assembly will be used to assemble the biobrick parts. Inserts containing promoters, RBS binding sequences, genes of interest, and terminators will be obtained within a *pSB1C3* or *pSB1A2* plasmid and will be digested using the *EcoR1* and the *SpeI* digestion enzymes. Each of these parts will be inserted into a cut plasmid digested with the *EcoR1* and the *Xba1* digestion enzymes. The desired insert and the cut plasmid will then be isolated via gel electrophoresis and purified via DNA extraction. Gel electrophoresis is performed, ensuring cut inserts and plasmid backbones of interest are of proper length and cut at the correct restriction sites. The insert and the cut plasmid backbone will then be mixed and ligated to join the parts together using T4 DNA ligase. After all, parts have been inserted,

extraction and PCR amplification of the entire gene will be performed from the plasmid backbone. After amplification, this new gene of interest will then be ligated into our plasmid of interest that will be used to transform *Sinorhizobium fredii*, our bacteria of choice.

A set of six different experiments will be run to test the validity of the solution. Firstly, *GFP's* release will be tested in the presence of GP. Another test will be performed to confirm that *GFP* isn't released in the absence of GP. This validates if *GFP* will act as an indicator of GP. Both one and two will also validate the expression of the *TrpR* (*PtrP* repressor). In the third experiment, the growth of the bacteria will be tested. The bacteria with the glpA gene is expected to grow. This is because the glpA gene confers that the bacteria is resistant to GP [7]. Another experiment will be done to test that bacteria without *glpA* gene expression dies. Afterwards, bacteria without the *glpB* gene will be grown in the presence of GP to test for GP breakdown. The last experiment is to ensure that the glowing is turned off when the GP is broken down in the presence of bacteria with the *glpB* gene.

Results and Discussion:

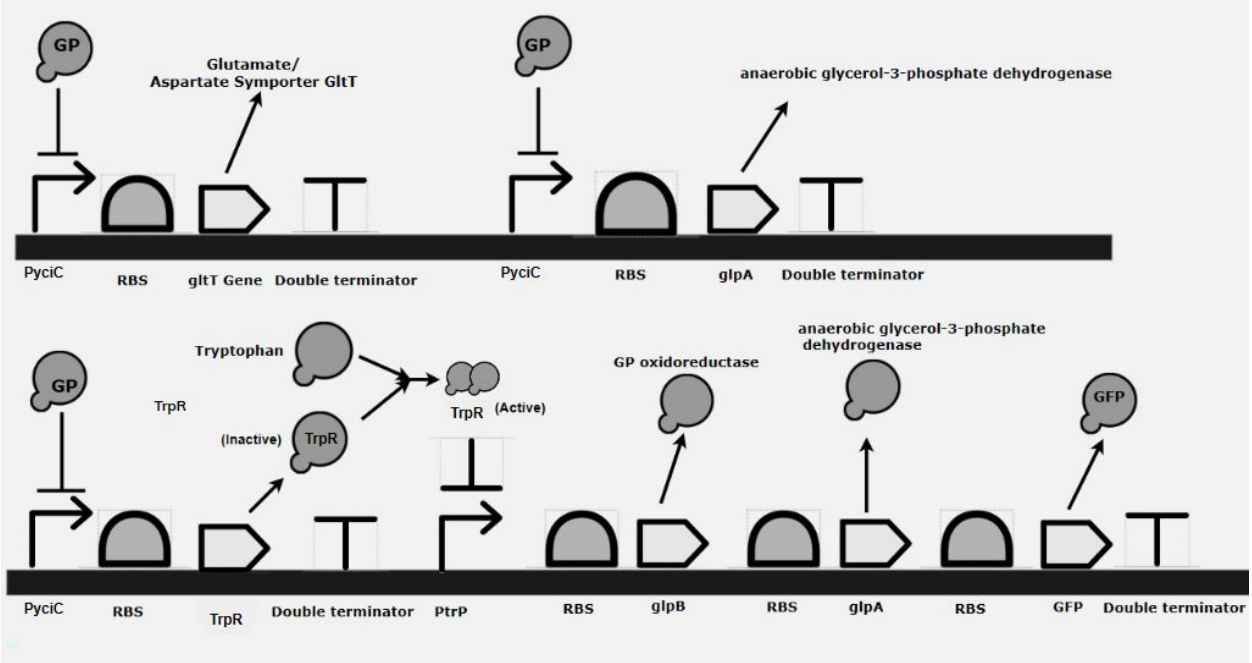

Figure 1. SBOL schematic for genes of interest; GP stands for glyphosate

A *PyciC* promoter, which is a glyphosate-regulated promoter [15], is promoting the *gltT* gene. The *gltT* gene releases glutamate/aspartate symporter *gltT* to move GP from the extracellular space into the cell. With GP bound to *PyciC*, the production of *gltT* is inhibited and the active transport of GP into the cell is stopped. *PyciC* also promotes *glpA*, which releases anaerobic glycerol-3-phosphate dehydrogenase to provide resistance to GP. Similarly, the presence of GP, and the repression of *PyciC*, inhibits the production of *TrpR*. *TrpR* is an inactive repressor. It needs to bind to tryptophan to be activated. Once it is activated it will repress the promoter *PtrP*. Once *PtrP* is activated, *glpB* will start to be expressed, which leads to the production of GP oxidoreductase. GP oxidoreductase will break down glyphosate inside the cell. *PtrP* will also promote *glpA*, so when *TrpR* is not present *glpA* will produce anaerobic glycerol-3-phosphate dehydrogenase. This will essentially make the cell resistant to glyphosate at all times. Lastly, *PtrP* promotes *GFP*, so when *TrpR* is not present *GFP* is produced.

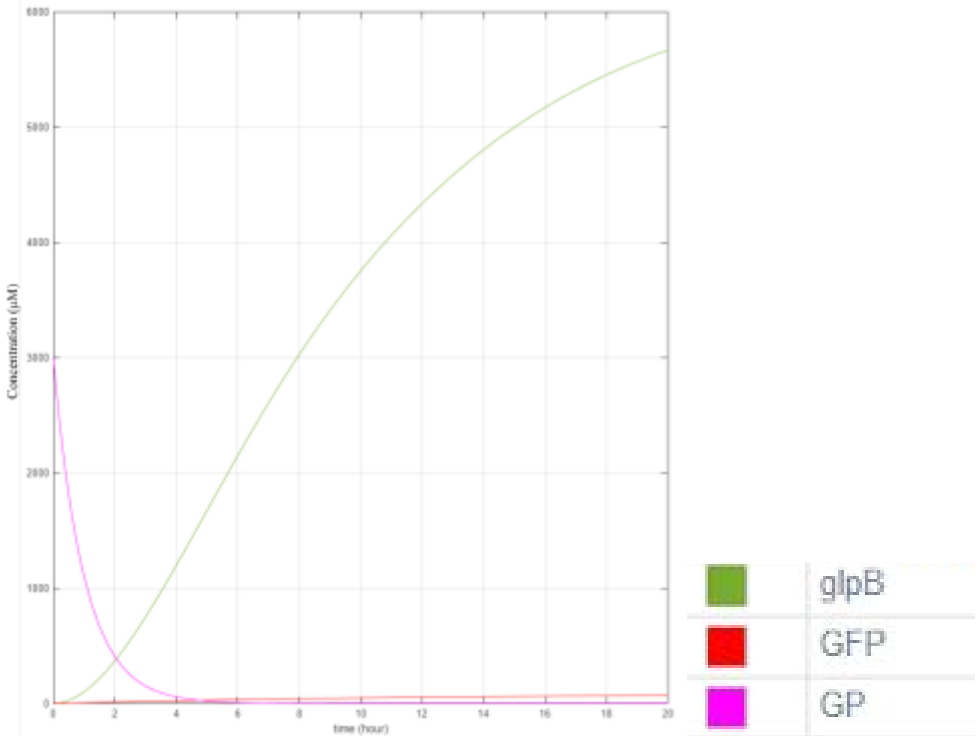

Figure 2. Expression of *GFP* and *glpB* gene with GP concentration over 20 hours

One goal of this system was to express *glpB* and *GFP* only in the presence of GP, and the loss of the repressor complex to the *PtrP* promoter region. Figure 2 shows how GP incorporated within the intracellular space affects the *GFP* signal and glpB gene expression. In an exposed intracellular environment, it is desired to show that as GP concentrations are present, *GFP* is being expressed and through the activity of *glpB*, GP concentrations would decrease. One flaw of this system was the lack of *GFP* expression in the simulation. This limitation can stem from our modeling data for the half-life of *GFP*, as well as the parameters for transcription and translation efficiency. Also, the concentration of *GFP* relative to concentrations of *GP* and *glpB* were much smaller, also indicating further thought into the settings for transcription and translation efficiencies under the *PtrP* promoter region, and the effectiveness of the *TrpR* repressor.

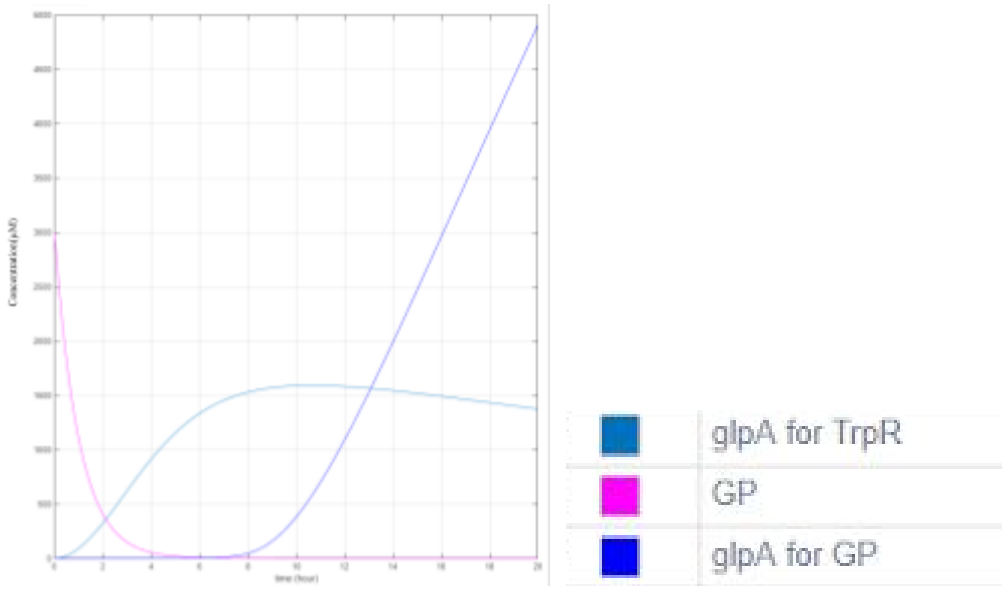

Figure 3. GP concentration and expression of *glpA* gene regulated under the *PyciC* promoter region under the presence of GP and regulated under the *TrpR* promoter region over 20 hours.

Another goal of the system was to maintain a constant expression of the *glpA* gene and constant GP resistance. This also makes the bacteria tolerant in the cases that could lead to transcription or translation errors that would otherwise threaten bacterial survival. From figure 3, the presence of GP has been shown to affect the expression of the *glpA* gene. The results show that with the initial presence of GP, *glpA* expressed under the PtrP is expressed, indicated by the light blue line. After approximately 6-8 hours, *glpA* is still continually expressed, however it increases to much higher levels indicated by the darker blue line (*glpA* for *TrpR*). While it is desired to maintain a constant expression of *glpA*, these results are limited based on the kinetic parameters specifically with *TrpR* as the repressor which increases at an exponential rate and is not kinetically regulated. Because nothing was regulating the breakdown of *TrpR*, this might have led to this overexpression of *glpA*, which can be mitigated in the future with kinetic data for *TrpR* breakdown or transcription/translation efficiency.

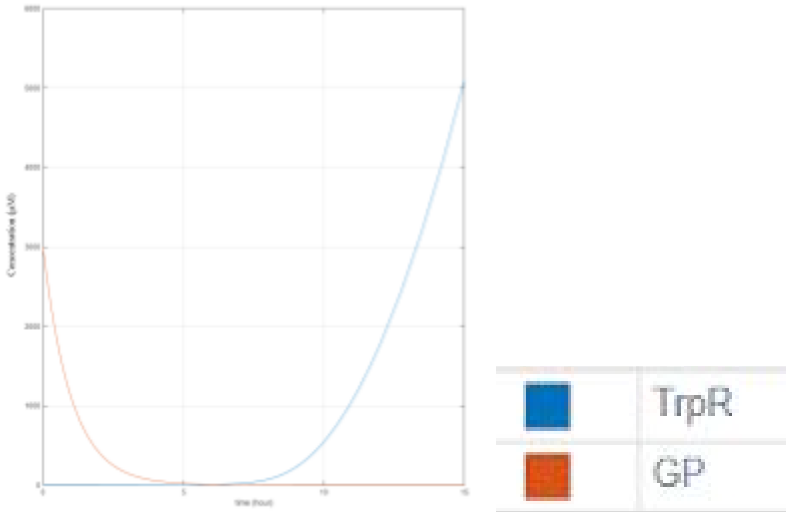

Figure 5. GP and *TrpR* concentrations over a 15 hour period.

A comparison of GP and *TrpR* concentrations was shown over 15 hours indicated in Figure 4. For up to approximately 5 hours, GP concentrations were noticeably present within the system compared to the concentration of *TrpR*. From our circuit diagram, it was desired to have GP act as the inhibitor to the *PyciC* promoter to prevent the transcription and further translation of the *TrpR*. The repression of *TrpR* prevents the complex formation with tryptophan that represses the PtrP promoter region. This would allow for the transcription and translation of the *glpB*, *glpA*, and *GFP* genes. Before approximately 5 hours, there was no presence of *TrpR* concentration showing that the presence of GP prevents *TrpR* from being transcribed and translated. From this observation, GP successfully inhibits the transcription and translation of *TrpR*. This was desired as the related genes of *glpB* and *GFP* that are repressed by *TrpR* should only be expressed in the presence of GP.

Conclusion:

In conclusion, the biological circuit successfully demonstrates the expression of the signal from the input, which is the presence of glyphosate, to the output which is the degradation of glyphosate and phosphorescence via *GFP*. The model uses *TrpR* and tryptophan to propagate the signal to the outputs.

The project also had many limitations in its design, one the biggest being the half-life of *GFP*. *GFP* has quite a long half life at around 54 hours so even after glyphosate breakdown has stopped *GFP* will be present in detectable quantities for many days afterward. This would result in the user having to wait longer to harvest than what is required. Another big limitation of the project is the assumption of rates and values. Due to many of the genes being used in this project being very niche, it was difficult to obtain the values for specific rates. To better accurately use Matlab Simbiology to model this system, greater accuracy can be achieved through obtaining kinetic parameters for *GFP*, GP intake, and *TrpR* expression.

In the future steps would be taken to ensure our product works efficiently and covers a larger plant population. Plant models would be used to test the effectiveness of glyphosate uptake and resistance mechanisms. There would also be an increase in the versatility of the product via an increase in inputs and outputs to cater to more pesticides. The same increase would occur in the output end with the breakdown of multiple pesticides and the use of different colour *GFP*'s to indicate the breakdown of each pesticide.

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Supplementary Information:

Table 1. List of BioBrick Parts Available from iGem

Part	Biological Part	Plasmid Backbone	Resistances
BBa_K2586001 [5]	*gltT* gene	pSB1C3 [13]	Chloramphenicol [13]
BBa_K2586008 [6]	Ribosome Binding Site (RBS)	pSB1C3 [13]	Chloramphenicol [13]
BBa_K2955001 [7]	*glpA* gene	pSB1C3 [13]	Chloramphenicol [13]
BBa_K2955002 [8]	*glpB* gene	pSB1C3 [13]	Chloramphenicol [13]
BBa_E0040 [9]	*GFP*	pSB1A2 [14]	Ampicillin [14]
BBa_K588000 [10]	*TrpR*	pSB1C3 [13]	Chloramphenicol [13]
BBa_K1592020 [11]	*Ptrp*	pSB1C3 [13]	Chloramphenicol [13]
BBa_B0015 [12]	Double Terminator	pSB1C3 [13]	Chloramphenicol [13]