Molecular Device to Biodegrade Pesticides, and Controlled by a RNA Switch

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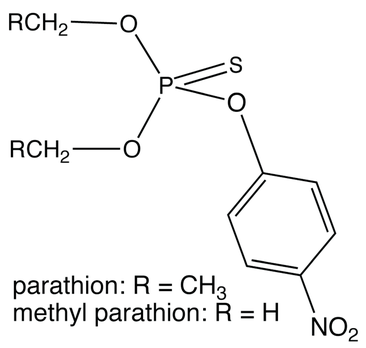
Abstract

Pesticides are commonly used in many countries around the world including Colombia and other Latin American countries. However, some pesticides are very stable in their chemical structures and take years to break down into less toxic forms. In many cases, they are also difficult to remove biologically. pesticides are less toxic, only when they can be degraded to the lower and/or single chemical stage. this process is known as mineralization. unfortunately, conventional methods are not effective to achieve this task. therefore, our aim is to degrade any pesticide to the least toxic stage through mineralization using different devices, and controlled by rna switch.

When humans use pesticides on crops, even if used in very small quantities, the pesticides accumulate in increasing proportions causing damages to other organisms present and eventually, to the higher stages of the food chain, affecting humans. Pesticides in humans can cause cancer, even years after being exposed to them, and they can also be fatal.
Organophosphates are one of the most common types of pesticides. They are widely extended and are used not only in farming, but also in households, in several industries and even as chemical weapons. The incorrect use of these pesticides is responsible for a great number of acute poisoning characterized by the development of cholinergic syndrome and multiple chronic complications, being neuropathy one of the most representatives. This type of chemical kills insects by disrupting their nervous systems. Furthermore, they can also harm the animal and human nervous system.

In humans, organophosphates add a phosphate to the active site of the acetylcholinestarase enzyme, thus, inhibiting its activity and preventing it from hydrolyzing acetylcholine. This causes acetylcholine to accumulate in the peripheral and central parts of the neurons as well as in the nerve endings, preventing synapses and inducing exited states in cells and organs.

Organophosphates are characterized by a double bond between phosphorus and oxygen, and are composed by different halogens, and an aromatic chain which makes them highly toxic. They are very unstable on an alkaline Ph, and they oxidize easily at the environment. The mechanisms of biotransformation of organophosphates are very varied and some of them can make a substance more or less toxic. For example, the oxidative parathion at the endoplasmic reticulum; on this reaction the phosphoric group increases the phosphorylating capacity of the parathion making it more toxic.



Chemical Estructure of parathion Presents a double bond between phosphorus and oxygen and a benzene ring

Our project is aimed at creating a device that is able to break down this type of pesticide, achieving their mineralization, with the intent of improving the environment.

There are different processes, such as the bioremedation and biodegradation that employ enzymes, microorganisms, fungi or plants to return a natural environment that has been altered by contaminants to its original condition. However, these processes require a rigorous control and its results can still be toxic substances.
This is why our device will reach the full mineralization of organophosphate pesticides and the improvement of the environment which has been contaminated with them.

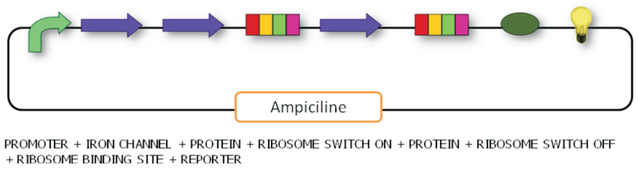
Objectives

1. use synthetic biology to design a device capable of breaking down organophosphate pesticides to the least toxic stage trhough mineralization, and controlling the protein mechanism in the device by using a rna switch, with the use of proteins derived from leguminosarum and pseudomonas putida.

2. Apply a computational model and an E-nose system to analyze and further predict the effectiveness of the device and the results of laboratory experiments.

Methodology

1. Construction and assembling of parts:



2. Construction of new parts

In order to achieve our objectives, we designed a device composed mainly of a Rhizobium leguminosarum protein. This protein produces phosphotriesterase, an enzyme that can break down the link between phosphorus and oxygen, a critical part of breaking down the pesticides.
We also used a Pseudomonas putida protein that acts on the remaining molecules and achieves their mineralization.

The gene sequences along with the standard parts from the MIT biobrick were constructed and assembled in vector pBSK II into a an E. coli chassis (E. coli, competent cells JM109 PROMEGA).
Sequences for the Rhizobium leguminosarum protein (GS44655-4 prl9), the Pseudomonas putida protein (GS44722 Xy1R) and the Promoter T3 (GS43581-1) were used. The riboswitch on and off, ribosome binding site and the sequences for yellow and cyan reporters were also assembled into the device. Enzyme digestions, ligations and trasformations were carried out according the instruction by PROMEGA.

3. Constructtion andassembling of device

Bacterial cells were transformed with pBSK II vector, with promoters (T3 and T7), and a Lac1 regulated device coding a monomerics yellow and cyan fluorescence proteins as reporters.
We started by performing an extraction of the plasmids containing the proteins, followed by a digestion of the sequences of Rhizobium leguminosarum with the restriction enzymes HindIII and BamHI, and pseudomonas putida with BamHI y EcorI using Promega protocol. At this point we faced a problem with a mismatch between the restriction sites and the vector used. Therefore, we decided to create specific oligonucleotide sequences which create artificial restriction sites through PCR.

Once we had the right restriction sites, we digested the pBSKII vectore and proceeded to ligate the sequences of Rhizobium leguminosarum and pseudomonas putida into the vector.
Finally, we introduce the remaining parts, such as the riboswitch (on and off), the ion channel proteins and the ribosome binding site.

The combination of these sequences allows for the break down of the organophosphate pesticides. Ultimately, the mineralization of these chemicals and their transformation into a harmless product is the main goal.

Transformed cells were tested in agarose gel with ampicillin to check the successfully process.

The viability of the device was also confirmed by the decrease in pesticide concentration, along with DNA fluorescence, the reporter protein, the ATP, and bacterium growth.

The decrease in pesticide was tested against different concentrations of organophosphates pesticides (0 – 0.5 – 1 – 5 µl), using e-nose to prove the decrease.

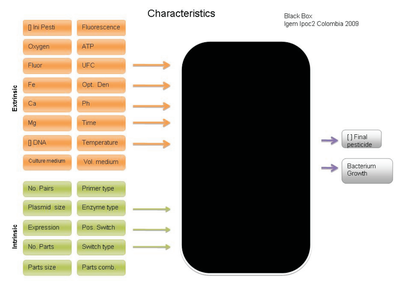
Computational Modeling

For this project we decided to use a neural network and an e-Nose(ELECTRONIC NOSE) system for our computational model. A neural network is capable of modeling relationships among data by learning from examples system, it is also able to relate multiple variables, and identify different factors, in order to predict the effectiveness of the device and the results of laboratory experiments.

The E-Nose system is an analytic device originally used for detecting chemicals and their concentrations in vapors. We applied this system to our project by finding the functional relationship between the concentrations of factors such as fluorescence, ATP, phosphotriesterase enzyme, DNA, DNA-polymerase and pesticide.

We introduce different kinds of organophosphate pesticides in different concentrations to train the model, making an identification of each sample.

After the model had been trained, we used it to identify the relation between the pesticide concentrations and DNA.



The fundamental e-Nose algorithm relies on the following equations:

$$r_{ij} = f_{ij}(c_j)$$

$$\bar{c}_j = \frac{1}{m}\left(c_{1j} + c_{2j} + \ldots + c_{mj}\right)$$

$$\sigma_j = \frac{1}{m-1}\left[\left(c_{1j} - \bar{c}_j\right) + \left(c_{2j} - \bar{c}_j\right) + \ldots + \left(c_{mj} - \bar{c}_j\right)\right]^{\cdot}$$

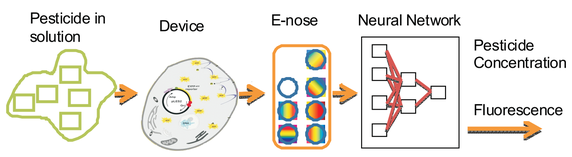
where

$r_i = f_{ij}(c_{ij})$

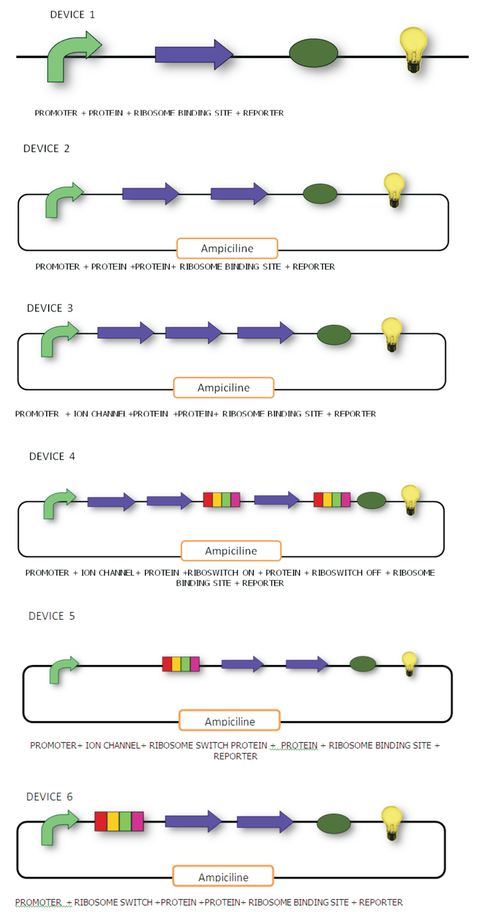
r_i = *response of species i as a function of concentration*

c_{ij} = *concentration of species i in* j^{th} *sensor*

Integration of the biological and computational models



Results – Constructed Devices



Conclusions

1. The device was assembled effectively.
2. The device was able to detect different moeities of the pesticide, that are suitable targets for degradation
3. The neural network computational system and the e-nose, show the best assembling of parts and device for degradation of the pesticide. this work is underway.