Lead detection and elimination in paint using fluorescent transformed *E. coli* and ethylenediaminetetraacetic acid

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For many homes built before 1978, lead paint was commonly used, especially between 1940–1959 (69% of homes) and before 1940 (87% of homes). Although many new products have arisen to combat this threat, including chemical-based indicators and lead treatment paints, one of the worst threats from lead paint is when it is sanded or removed. We plan to design a biosensor which neutralizes the toxicity of lead dust and indicates the lead concentration remaining. To achieve this, a plasmid containing three recombinant genes will be transformed into *Escherichia coli* cells. These recombinant genes will act as a circuit where the first constitutive gene will produce lead-sensing proteins. If lead is present, these proteins will bind to the second gene's promoter, causing it to produce GFP and LacI proportionally to the amount of lead in the cell. The third gene will be repressed using LacI and will produce mKate2, a red fluorescent protein, which will signal a lack of lead. Next, a solution containing Ethylenediaminetetraacetic acid (EDTA) will be prepared to neutralize the negative effects of the lead. EDTA bonds with metal ions and eliminates their functionalities within most biological cells. The transformed red-expressing *E. coli* will be added to Eppendorf tubes. Samples from locations on a painted surface will be added to the tubes to test. If lead is present, the *E. coli* will begin fluorescing green and the EDTA solution will be applied to the surface. EDTA will begin to bond to the lead in the paint, decreasing the amount of unbound lead present. After application, the surface will be retested to confirm that there is no more toxic lead.

Keywords: Lead detection, EDTA, *Escherichia coli*, mKate2

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Watch a video introduction by the authors at https://youtu.be/zUYDWZ2IEfY

Background

Lead paint is a major health concern for everyone exposed to it. Historically, it was commonly used because it is soft and malleable whilst also not being electrically conductive (Wani, Ara and Usmani 2015). Unfortunately, these users of lead paint were either notconcerned with or unaware of the accumulation of its particles within the environment and its toxic nature. Because lead is not biodegradable, it remains toxic in large quantities over large periods of time (Wani, Ara and Usmani 2015). One of the worst threats from lead paint is when it is sanded or removed, as leaded paint dust gets into the air, allowing it to drift into the lungs

of nearby living creatures and spread throughout the environment.

The dangers of lead exposure to living creatures include development of cognitive disabilities, decrease in function of the nervous system, blood disorders, and severe damage to the brain and kidneys (Wani, Ara and Usmani 2015). In humans, it enters the cell by destabilizing the cell membrane using lipid peroxidation (Kim, Jang, Chae, et al. 2015). After entering the cell, lead causes cell death within the human body by oxidative stress (Flora, Gupta and Tiwari 2012). Oxidative stress is the result of an overabundance of free radicals relative to the biological system's ability to detoxify the reactive intermediates or to repair the damages inherited as a byproduct of the aforementioned free radicals (Flora, Gupta and Tiwari 2012). In the case of lead induced oxidative stress, lead binds to and inactivates an antioxidant called glutathione (Kim, Jang, Chae, et al. 2015) which would normally be responsible for regulating free radicals in the cell. Once lead enters the cell, glutathione is incapable of regulating those free radicals due to lead's high affinity for glutathione. Lead is also able to substitute itself for other important cations within the cell, such as calcium, magnesium, iron, and sodium, which can shut down key communications within the cell and lead to irregularities in protein folding, release of neurotransmitters, and apoptosis of the cell (Flora, Gupta and Tiwari 2012).

Unfortunately for the people who have had high exposure to lead, lead has a near-permanent effect on the human body (Flora, Gupta and Tiwari 2012). In the realm of lead poisoning treatments, the use of chelating agents, such as EDTA has been recommended in cases of acute exposure, however is detrimental to chronic cases. Otherwise, there has been little discovered in the realm of lead poisoning treatments, meaning the best option available is to avoid prolonged exposure to lead products. This is, in theory, easy to accomplish, except when demolishing a lead-painted house.

Although there are paints available to treat lead-painted surfaces, such as encapsulants that trap the lead in the wall but don't treat it, it would be wasteful to buy gallons of it and spend the time needed to apply it only to tear down the walls the treatment has been applied to. Also, encapsulants do not prevent lead from becoming aerosolized in demolition. Using the chelate Ethylenediaminetetraacetic acid, or EDTA is a possible alternative. Dissolving EDTA in an aqueous solution and applying it to a lead painted surface should neutralize the lead when the EDTA binds with the lead in the paint.

EDTA is used as an agent in cosmetic applications, food preservatives, and the removal of heavy metals, including lead, in plants, animals, and humans. For example, a study by the International Agency for Research on Cancer (IARC) on inorganic and organic lead compounds, notes a process called phytoextraction, in which plants can pull heavy metals out of the soil through their roots and then store it in their stems. While this process is usually slow, the presence of EDTA accelerates the translocation of lead in the stem while also reducing toxicity (International Agency for Research on Cancer 2006). EDTA, as mentioned previously, is also used in chelation therapy, to treat lead poisoning.

The second part of the project provides a color–coded test as to the amount of lead contained in the painted surface. *E. coli* transformed with a lead sensing promoter will respond to concentrations of lead by producing the green-fluorescent protein GFP. When there is no lead present, the *E. coli* will express the red-fluorescent protein mKate2.

Systems level

As a system, the parts work to detect lead in the surrounding environment. The entire project serves as a test to identify the presence of lead in painted surfaces. To that end, two systems are used to test. The first is a solution containing transformed *E. coli*, which will be used to take measurements of lead content in the paint. In the example of testing a wall for the presence of lead, tests are conducted using paint chips taken from the surface of the wall at multiple locations. The chips are then placed into Eppendorf tubes, where lead free radicals in the paint will bind with the lead sensing protein in the *E. coli*. Depending on the lead concentration, the *E. coli* will either produce red (indicates that no lead is present) or green (indicates lead is present) through the use of fluorescent proteins.

If lead is not present in any of the samples, the surface is clear to be safely demolished without risking aerosolizing lead dust in the process. If lead is present, the process will move onto the second stage, which is the reduction of lead ions present in the paint. A solution of EDTA will be applied to the surface. Once it is applied, the EDTA will be given time to bind to the lead. EDTA, a chelate, readily binds to metal ions and prevents them from binding to other molecules. New paint chips will be tested in the *E. coli* solution to see if all of the lead has been chelated, which will be recognized by only having red fluorescent proteins present in the solution.

Device level

The *E. coli* will be transformed to express three genes (Figure 1). The first gene is constitutive and will always

be producing lead sensing proteins. If lead is present (Figure 2), the lead sensing proteins will bind to the promoter of the inducible second gene to produce GFP and LacI. The LacI produced from the second gene will repress the third gene, which produces mKate2, which is a red fluorescing protein that will express i lead is not present. After the *E. coli* has detected lead, then EDTA will be placed into the solution to neutralize the negative effects of lead. EDTA does this by bonding with the metal ions.

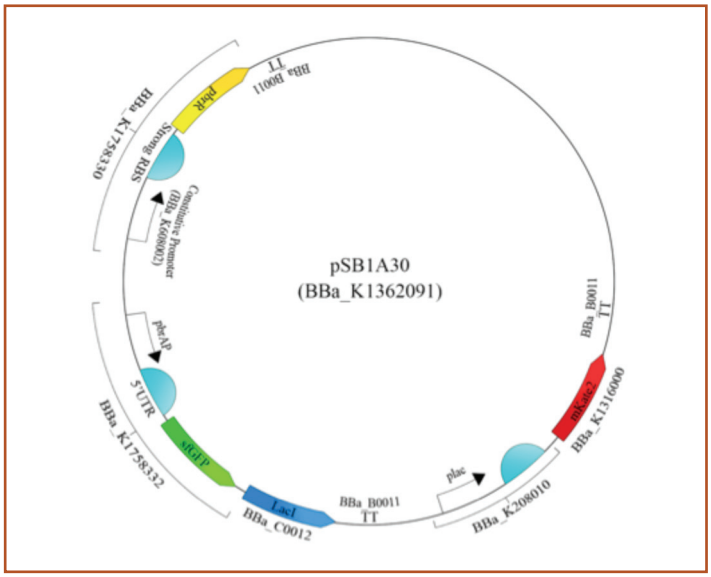

Figure 1. *The picture above represents the assembled plasmid, which will be transformed into the cell. The plasmid, on a basic level, causes the cell to fluoresce red normally and causes it to fluoresce green in the presence of lead.*

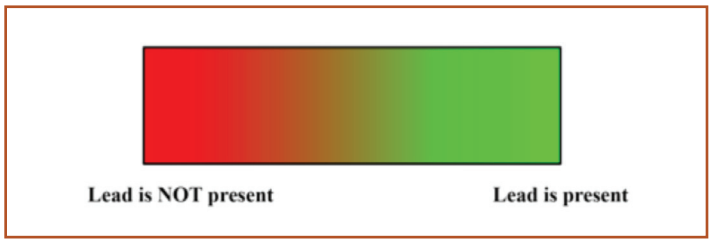

Figure 2. *The image above visually indicates the colors representing lead and those representing the absence of lead and the gradient between the two. Because of a lack of lab experience due to COVID-19, these colors may not be entirely accurate, but are instead an estimate.*

Parts level

The parts used were BBa_K1758330, a part of a lead sensing promoter system; BBa_K1758331, another part of the same system; BBa_K1758332, a part of the lead-sensing promoter system which expresses GFP in the presence of lead; BBa_C0012, LacI; BBa_K208010, the Lac operon's promoter and RBS; BBa_K1316000, a gene expressing mKate2; BBa_B0011, a bidirectional terminator; and BBa_K1362091, a high-copy backbone, carrying Amp resistance (Figure 3).

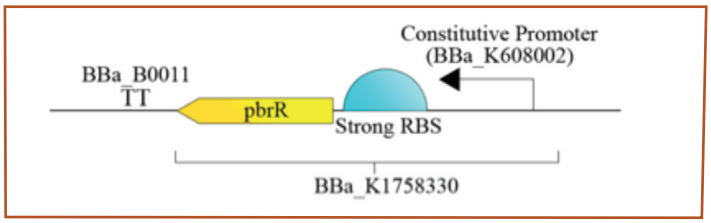

Figure 3. *This composite part expresses the protein that binds to lead to induce the second composite part of the system.*

The lead-sensing promoter system works by producing a protein which binds with lead and induces the expression of sfGFP (Figure 4). This entire system was developed and first designed and used by a team of researchers from Bielefeld University in 2015. The LacI gene will then be added to the lead-sensing promoter to express LacI in the presence of lead. This represses the expression of our other operon.

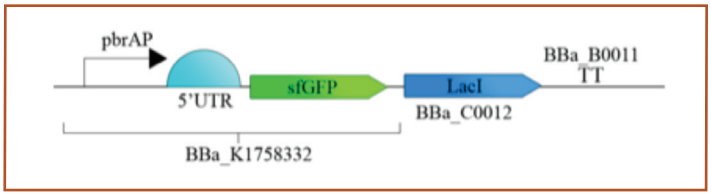

Figure 4. *The above image displays the second composite part within the Renaissance lab's lead sensing system. This part produces GFP to signal the presence of lead to the user.*

The other operon will use the Lac promoter to constitutively express mKate2, which is a red fluorescing protein that will express if lead is not present (Figure 5). This will cause the expression of mKate2 to halt once lead is detected in the cell environment. The amp resistance was chosen as a selective marker for the successfully transformed bacteria.

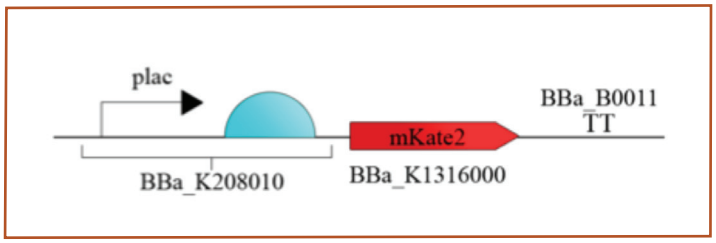

Figure 5. *The final composite part, which uses the promoter from the lac operon and expresses mKate2, a red fluorescent protein, causing the cell to constitutively fluoresce red, aside from when it is repressed due to the presence of lead.*

In combination, the first composite part works with the second to detect lead, producing sfGFP and LacI if there is lead present within the cell. Due to the third composite part, mKate2 is constitutively expressed within the cell, causing it to fluoresce red. This expression of mKate2 is repressed using LacI, causing the cell to stop fluorescing red when in the presence of lead. Because the second composite part expresses sfGFP as it represses the third composite part, the cell then fluoresces green to indicate the presence of lead.

Safety

There are potential health risks with the EDTA and any lead in the paint. EDTA has been ruled safe by the Cosmetic Ingredient Review Expert Panel. However, EDTA can be toxic when ingested which can have reproductive and developmental effects. The lowest dose that is possible to cause toxic effects is 750 mg/kg/day (Lanigan and Yamarik 2002). EDTA is unlikely to be absorbed through the skin, but there is a possibility that it can affect other chemicals into the skin. One of the biggest dangers with overexposure to lead is that it can disrupt the functioning of brain neurotransmitters. Symptoms of overexposure to lead can range from headaches, irritability, abdominal pain to anemia, changes in kidney function, seizures, coma, and death (Farley 1998). The system will be safely constructed using proper lab techniques and sterile lab procedures. The transformation of OP50 *E. coli* will be completed in a controlled lab environment. Only approximately one square centimeter of paint will be used to test for lead. Thus, it will ensure that there is no risk of being harmed by any potential lead in the paint. The same is valid for the EDTA, only the amount needed will be used. Both paint and EDTA will be used in moderation. The selective marker that will be included in the plasmid will be an Amp resistance. This means that the *E. coli* solution will be able

Discussions

The main benefit of this project would be to provide a method of efficiently neutralizing lead dust with minimal environmental risk involved. The possibility of chelating the lead with EDTA may mitigate the risk of lead exposure, while providing a robust testing method ensures that at risk workers take necessary precautions.

The challenges of this project include successfully transforming the chosen *E. coli*, getting into the lab to perform the necessary procedures for the experiment, ensuring that the *E. col*i survive long enough to be useful for testing, and making sure that too large of a dose of lead does not immediately lyse the *E. coli*. Successfully transforming the *E. coli* should prove difficult because the plasmid our lab will be producing is rather large. Evidently, the coding regions could be divided into two separate sections and transformed into the cell as two separate plasmids, however because the plan for this project is to distribute it to the public, giving the bacteria resistance to more than one antibiotic is not advisable.

Many aspects of this project still need to be determined for it to be a finished product. Most of them would be resolved in a lab setting with the ability to repeatedly test and revise our *E. coli* and EDTA solutions. First and foremost, the concentrations of EDTA and other ingredients in the solution need to be determined experimentally. Given that EDTA binds with lead in a 1 to 1 ratio, and that the federal definition of lead paint is 1mg of lead per centimeter squared, it can be expected that 1.4mg of EDTA per square centimeter (or ~130g per 100 sq ft) is required to bind the lead.

We recognize that in quantities over 750mg/kg/day of EDTA can be toxic (Lanigan and Yamarik 2002), since EDTA will chelate important metals like calcium in people and animals. While the expected amount of EDTA required to cover a large painted surface is well in excess of that quantity, there are two main factors to consider. One, it would be difficult to receive a fatal dose of EDTA through its application to the wall, especially when it's painted on, as EDTA isn't absorbed through the skin (Lanigan and Yamarik 2002). Secondly, the stability constant of the lead-EDTA complex, an 18.3, is the second highest of EDTA-metal complexes, with nickel-EDTA having a constant of 18.4, and the next highest (Cd-EDTA) having a constant of 16.4 (Flora and Pachauri 2010). This means that once EDTA binds to lead, most other metals, particularly Calcium, Sodium, and Iron, are unable to replace lead in the complex. As such, once lead and EDTA bind on the painted surface, neither is toxic to humans. This significantly reduces the likelihood of receiving a toxic dose of EDTA from the treated surface.

Other considerations for this project include taking into account the viscosity of the solution so that it doesn't completely run off of a vertical surface, how well the solution can permeate painted surfaces, how long it will take to react with the lead, how quickly the solution dries, and other factors. Likewise, the ability for the *E. coli* solution to detect lead still bound in paint will need to be tested, along with the sensitivity of the lead-sensing promoter.

Ecological concerns will also have to be addressed. While lead-bound EDTA isn't harmful to humans, it can still penetrate soil. Making sure the *E. coli* solution is handled safely to avoid exposure to the environment or other people is also crucial, making it necessary to find a better solution for the general public.

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