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Design Brief

Detox-i-Fish: novel approach for an *Escherichia coli* based solution that reduces methylmercury in fish*

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Our project aims to combat mercury contamination and mercury poisoning in food. Mercury poisoning is a lethal condition which can affect brain development when not addressed and is especially prevalent in coastal communities and fish reliant countries. We are working on developing an Escherichia coli plasmid that uses different Mer proteins to break down methylmercury in fish into less toxic elemental mercury. We use four sets of Mer proteins linked together that act as a transport and enzymatic system. We plan to soak raw fish in this E. coli solution in order to break down the methylmercury into elemental mercury. Then, we will heat them to remove all the E. coli, which cannot survive in higher temperatures. When fish consume elemental mercury, methylmercury is created as a byproduct of the bacteria naturally found in fish. These bacteria only exist in the ocean, so if methylmercury is converted back into elemental mercury after the fish are removed from the ocean, the mercury will remain in the less toxic elemental state, posing less harm to humans. This plan will be an efficient and effective treatment process to ensure food safety. A potential concern is the methane byproduct created from the Mer proteins. While the concentration of the methane produced is negligible, we still aim to make the process as safe as possible and want to research how to minimize or remove the methane. For proof of concept, we wish to prepare fish samples containing methylmercury at different depths into the skin, place the fish into different E. coli solutions and check for the methylmercury concentration in each of the fish and record to see where the penetration levels out. We also want to contact companies that make canned fish and talk to them about how we can implement this idea.

Keywords: Mercury poisoning, Mer proteins, methylmercury, elemental mercury, Detox-i-Fish

Biomagnification is the accumulation of toxins in an organism due to it ingesting other organisms with high concentrations of those toxins. Biomagnification of materials such as heavy metals has always been an issue, and one example of this is the biomagnification of mercury in fish. Sixty-nine studies of one hundred twenty-nine food webs confirm that mercury biomagnification in marine ecosystems is steadily worsening (Lavoie et al, 2013). This is an issue for people because mercury is toxic in high concentrations and could jeopardize brain development as seen in Figure 1 (Nogora, et al. 2019). In 2017, the average American consumed sixteen pounds

* *The authors were mentored by Kristen Jenkins, Pam Seeley, and Catherine Sharer from Lambert High School. Please direct correspondence to: f19723@forsyth.k12.ga.us.* *This is an Open Access article, which was copyrighted by the authors and published by BioTreks in 2024. It is distributed under the terms of the* Creative Commons Attribution License*, which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited.*

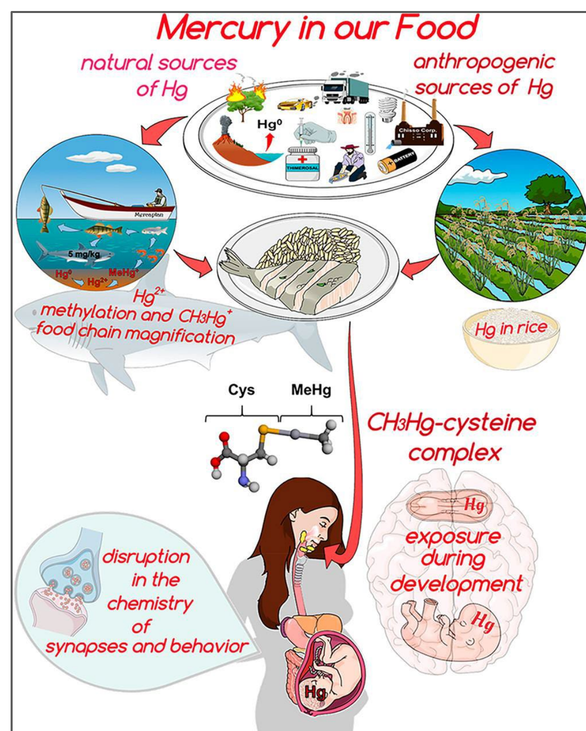

Figure 1. This shows an example of how mercury contamination has been affecting human lives and the side effects of contaminated food.

of fish and shellfish, the two largest reservoirs for mercury biomagnification, and that number is expected to increase annually (NOAA, 2018).

These groups of contaminated fish species are not only harmful to marine wildlife, but they end up harming us humans as well when they enter the supply chain through fisheries. These contaminated fish are caught up by large fishing nets in groups and then they are sent to factories to be processed and marketed as either fresh produce or canned goods. Canned tuna alone has produced over $2.7 billion in 2020 and has continued growing and this comes to around 540 million cans sold annually with around 1 billion pounds of tuna being eaten by Americans alone. (National Fisheries Institute, 2024; Watson, 2023). The global canned seafood market has come around to about 30.09 billion dollars in value and these are just a few of the statistics that describe the sheer size of the canned seafood market (Consumer F&B, 2021).

Due to the sheer size of the market as seen in Figure 2(NOAA 2021), a lot of problems may come as a result in any amount of change that damages ecosystems. This is why when biomagnification and mercury contamination are brought into the market they affect so many people that it becomes a worldwide problem and has to be addressed. If over 1 billion pounds of tuna are going out to American's and even if 0.1% of that is contaminated with mercury that is about 1 million pounds of mercury contaminated tuna being ingested by Americans. When in reality around 27% of fish are contaminated with more than the recommended limit. This also only accounts for tuna with all seafood combined. It shows the scale of the problem and why we need solutions as soon as possible.

While there have been some attempts to remove mercury out of our ecosystems and fish, they have fared unsuccessfully. This has even led large governmental organizations like the U.S. Environmental Protection Agency (EPA) to step in but even their efforts haven't been very successful. This is because large amounts of our fish come from outside the U.S. where the U.S. government cannot institute regulations and because of the scale at which the problem is at. Not only that, there is no cure for mercury poisoning. The only real way to deal with this problem is to either tamper with the marine wildlife directly or change the process at which the fish are sent out into the market. Our method for dealing with this problem comes from the latter of the two solutions as our implementation is in the canning process of the goods.

Mercury once entered into a fish will be hard to remove because it would have spread to multiple parts of the body and we don't have any current solutions that are able to remove the Mercury. However, it is possible to turn that mercury into a less harmful substance which will not cause mercury poisoning or other harmful conditions. This is also the method that we are using to approach this problem. Using a sequence of proteins and enzymes we are converting the methylmercury into a less toxic version of mercury which doesn't cause mercury poisoning. This would allow us to take out very large amounts of mercury and target mercury all around the parts of the fish.

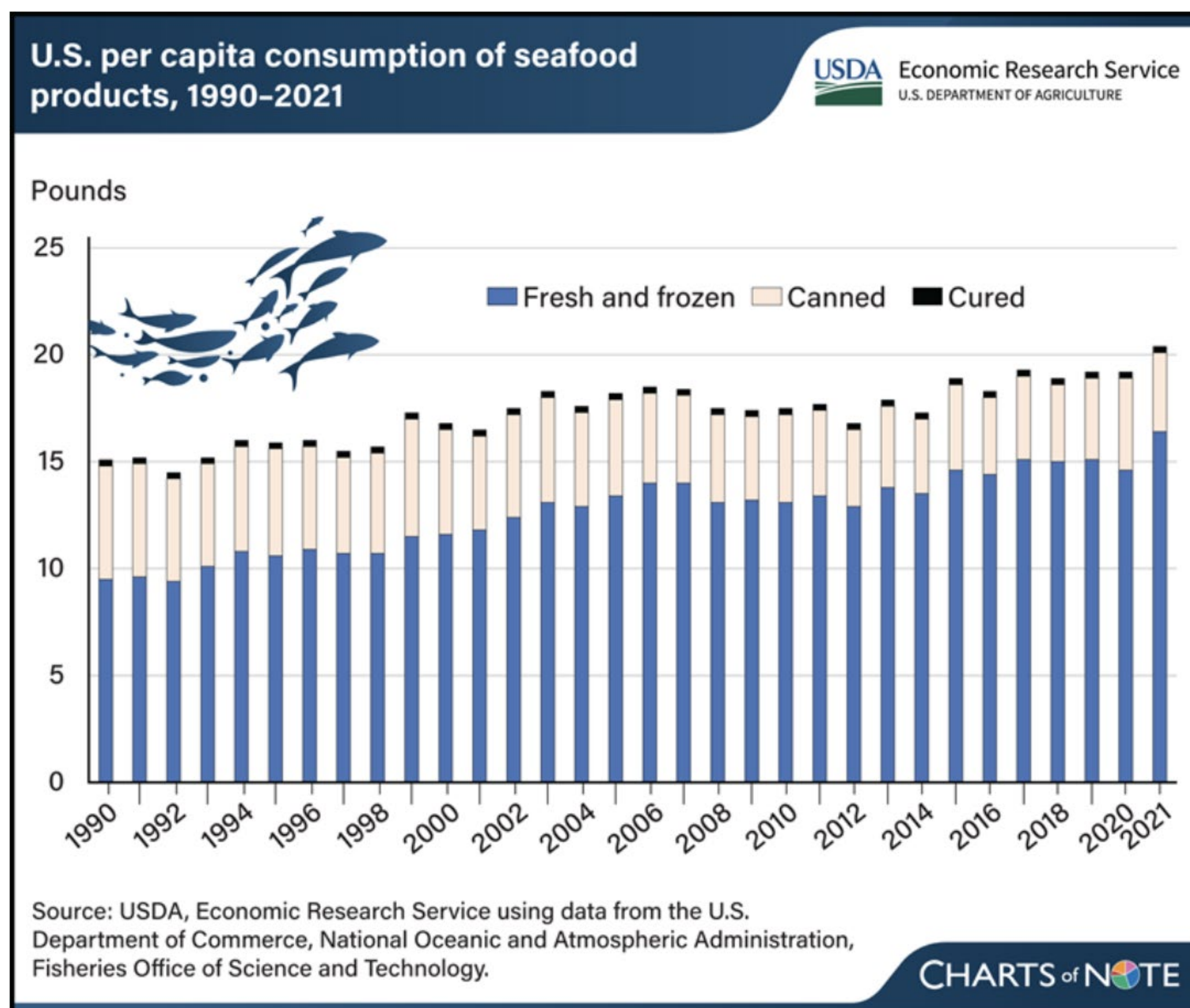

Figure 2. Data showing the size of the seafood market by category and growth in the future.

Systems level

In order to remove/remediate mercury in fish, we plan to create a plasmid that can be inserted into a strain of *E. coli* and make an *E. coli* solution for the fish to be placed in. Like mentioned before there is no current way to remove the mercury from the fish by simply cooking the fish, and groups such as the EPA are trying to reduce mercury production. However, there is not a very simple nor cost effective way currently to remove existing mercury in the fish. Canning companies especially are not implementing some existing methods, which leads to harmful effects when methyl mercury is consumed.

The Mer proteins inserted into the plasmid work together with each other to deliver and metabolize the mercury. By using the Mer proteins, we aim to effectively alleviate the toxic methylmercury from the fish by turning it into a much safer form, being elemental mercury. We plan to make this plasmid readily available for canning companies to use during their canning process, and we hope to spread its use to mitigate as much methylmercury as possible. Mer proteins are a set of proteins that deal with mercury in bacteria cells and allow bacteria cells to transfer and breakdown mercury. Mercury reductase is an enzyme complex of these proteins and these focus on the actual breakdown of the methylmercury

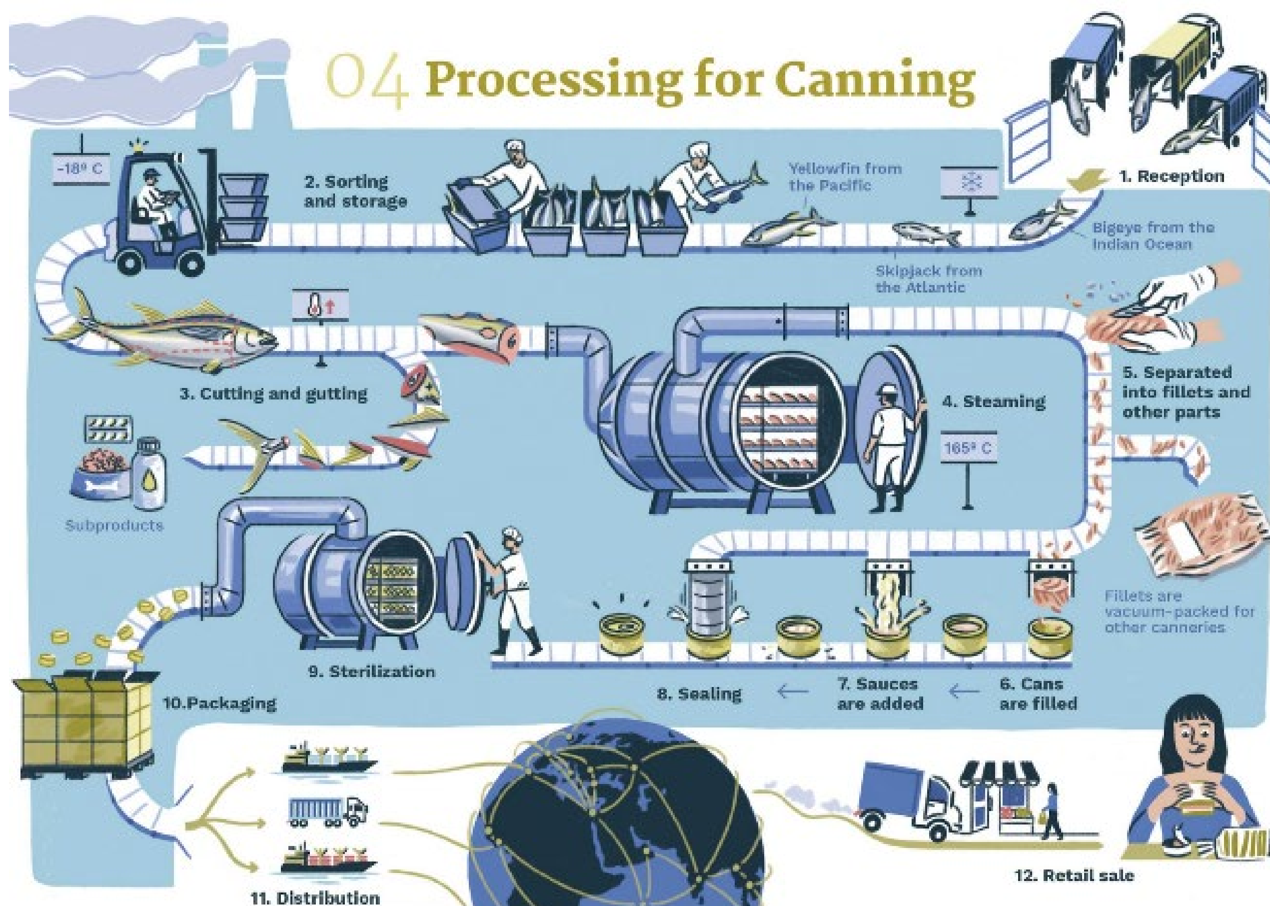

Figure 3. The current processing of canning fish after it is has been caught(Illustrations by Flavia Gargiulo for Planet Tuna)

into the less toxic version also known as elemental mercury. Mercury reductase uses NADPH as an electron donor to catalyze the reaction which makes methylmercury lose two electrons and become elemental mercury. The percent yield of mercury reductase is actually fairly high with about an 80% percent yield by itself (Fox, 2024). By studying mercury reductase we found the mechanisms of its reactions and these mechanisms work with several types of bacteria but the main one is *E. coli* and its efficacy is very high as seen by the percent yield.

However, mercury reductase proteins can't work by themselves as they have no real way of locating methylmercury towards them and getting to any mercury that they can convert and this is where mercury transport proteins come in. Mercury transport proteins are aptly named as they are proteins that transport mercury. They bring the mercury from around the cell towards the *E. coli* where the *E. coli* breaks it down using the mercury reductase and then this converts the mercury into a less toxic version. However, just one mercury transport and one mercury reductase won't work because each protein has their own niches and not all proteins have the same functions like some transport proteins may only target the cell membrane while others look inside the cytoplasm for mercury. This is why multiple layers of these proteins have to be included in order to be able to have a functioning system that actually deals with the mercury.

The Mer proteins we used are two mercury reductase proteins and two mercury transport proteins. The mercury reductase protein is MerA and another protein type called organomercurial lyase (MerB) work in cohesion to break down methylmercury very

efficiently and they have a feedback mechanism that allows them to work together and not over or underproduce for each other. The two mercury transport proteins are MerP and MerT which target different parts of the cell with MerP targeting the periplasmic part and MerT targeting the inner membrane and together they get to a large part of the cell which severely reduces the amount of methylmercury. This sequence of Mer proteins is able to reduce 97% of methylmercury after 10 hours of growth (Astolfi, 2016).

The most important part is the stage of the canning process where the *E. coli* plasmid will be used. Because the *E. coli* plasmid has certain conditions required to live, there are only a few spots where the implementation can actually occur. We believe that the best way to integrate our plasmid into the current system used for canning would be to add a step between steps 3 and 4 shown in Figure 3 (Planet Tuna) that includes a suspension system in which the *E. coli* plasmid and the fish will be put in. To ensure optimization for both mercury reduction and the canning companies, we propose for the fish and plasmid to be in a suspension which will allow for the *E. coli* to penetrate and detoxify the fish. To do this, we want to contact companies that make canned fish and talk to them about how we can actually implement this idea, or more specifically the step that we wish to add. From them, we can learn more about the process of canning fish in order to avoid creating any harmful effects of the product.

A possible suspension system could be designed with these features: The main component is a suspension tank made of stainless steel or another food-safe material to prevent contamination. The tank has to be large enough to fit the volume of fish being processed. It should also have an agitation system with stirring mechanisms or aeration to keep the *E. coli* solution evenly distributed and ensure thorough contact with the fish. Additionally, there should be an *E. coli* solution dispenser with a reservoir for the engineered *E. coli* solution, maintained under optimal conditions for *E. coli* viability. Automated pumps or valves can dispense the solution into the suspension tank at controlled rates, causing consistent concentration. The system would also include a fish loading and unloading mechanism with automated conveyor belts and timers to control the duration each batch of fish spends in the suspension.

Device level

The plasmid that we use is to be implemented inside of *E. coli*. We are using *E. coli* because its rapid reproduction allows for high volume amplification of plasmid copies, which is needed for wide-scale methylmercury reduction. Also, *E. coli* thrives on cheap and easily obtainable media, making it an easy to use and affordable option. Also, a deep understanding of *E. coli*'s genetics helps with plasmid manipulation and behavior prediction within the host. Finally, most commonly used strains lack viral factors, which ensure safety.

There are four main proteins used for methylmercury metabolism. These included MerA, MerB, MerP, and MerT. MerA arranges the enzyme mercuric ion reductase, which catalyzes the reduction of Hg(II) to its volatile and non-toxic form, Hg(0). The active site of the enzyme has four cysteine residues that work in mercury binding. The C-Terminal site is used for catching mercury from solutions and delivering the mercury to the core. This gene is very important to mercury resistance in bacteria and the cytosolic protein works with a NADPH molecule which is necessary for the power to reduce Hg(II) to Hg(0).

The process starts with the MerP protein which is a periplasmic mercury transporter, that captures mercury in both organic and inorganic forms and moves it to the membrane integrated protein, which is encoded by a MerT gene. The MerT protein then receives the mercury from the MerP protein at its first transmembrane helix. A pair of cysteine residues take the mercury and its sulfur atoms break the bonds between mercury and the other binders. MerB is then translated into organomercurial lyase, an enzyme that breaks the bonds between methylmercury and a radical, reducing the methylmercury into Hg(II), as seen in Figure 4. MerA then breaks Hg(II) into Hg(0), which is nontoxic elemental mercury, as seen in

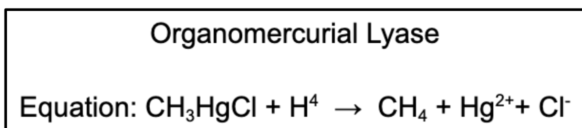

Figure 4. The transformation of methylmercury into Hg^{2+} and CH_4 (Methane) by the transformed MerB protein.

Figure 5.

The MerB protein is able to transform multiple types of methylmercury, such as normal methylmercury and methylmercury chloride. The general formula for methylmercury CH_3Hg^+, but there are different types of methylmercury such as methylmercury chloride which has the formula CH_3HgCl. The bonded chlorine is known in chemistry as a radical, or an atom, molecule, or ion with at least one unpaired valence electron. However, the MerB protein accounts for this as the R - shown in Figure 6 symbolizes any radical, meaning that the MerB protein will be able to break down most organomercurials like methylmercury, methylmercury chloride, ethylmercury ($C_2H_5Hg^+$), and dimethylmercury ($(CH_3)_2Hg$).

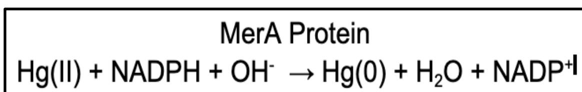

Figure 5. This shows the reaction equation as MerA turns the methylmercury into the elemental mercury.

The rest of the parts of the plasmid are more general parts, not specific to mercury detoxification. The T7 promoter, a strong starter recognized by an external enzyme (T7 RNA polymerase), controls the gene we want

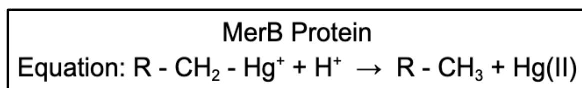

Figure 6. MerB transforming methylmercury with a radical into Hg(II).

to express. This expression is tightly regulated by the LacI repressor protein, which binds to the LacO sequence and blocks transcription when present. The RBS site on the resulting mRNA then attracts ribosomes to begin protein synthesis. The plasmid replicates because of the pBR322 ori, and the ampicillin resistance gene will allow us to identify bacteria that have taken up the plasmid during transformation by growing them on media containing the antibiotic ampicillin.

Parts level

The four main proteins used for methylmercury metabolism are MerA, MerB, MerP, and MerT, which can all be seen in Figure 7. MerA arranges the enzyme mercuric ion reductase, which catalyzes the reduction of Hg(II) to its volatile and less-toxic form, Hg(0). The active site of the enzyme has four cysteine residues that work in mercury binding. The C-Terminal site is used for catching mercury from solutions and delivering the mercury to the core. This gene is very important to mercury resistance in bacteria and the cytosolic protein works with a NADPH molecule which is necessary for the power to reduce Hg(II) to Hg(0).

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Safety

Considering the volatile and dangerous nature of mercury and *E. coli*, safety will be a very important concern.

Because of the adverse effects of *E. coli*, it is necessary to make sure that it does not

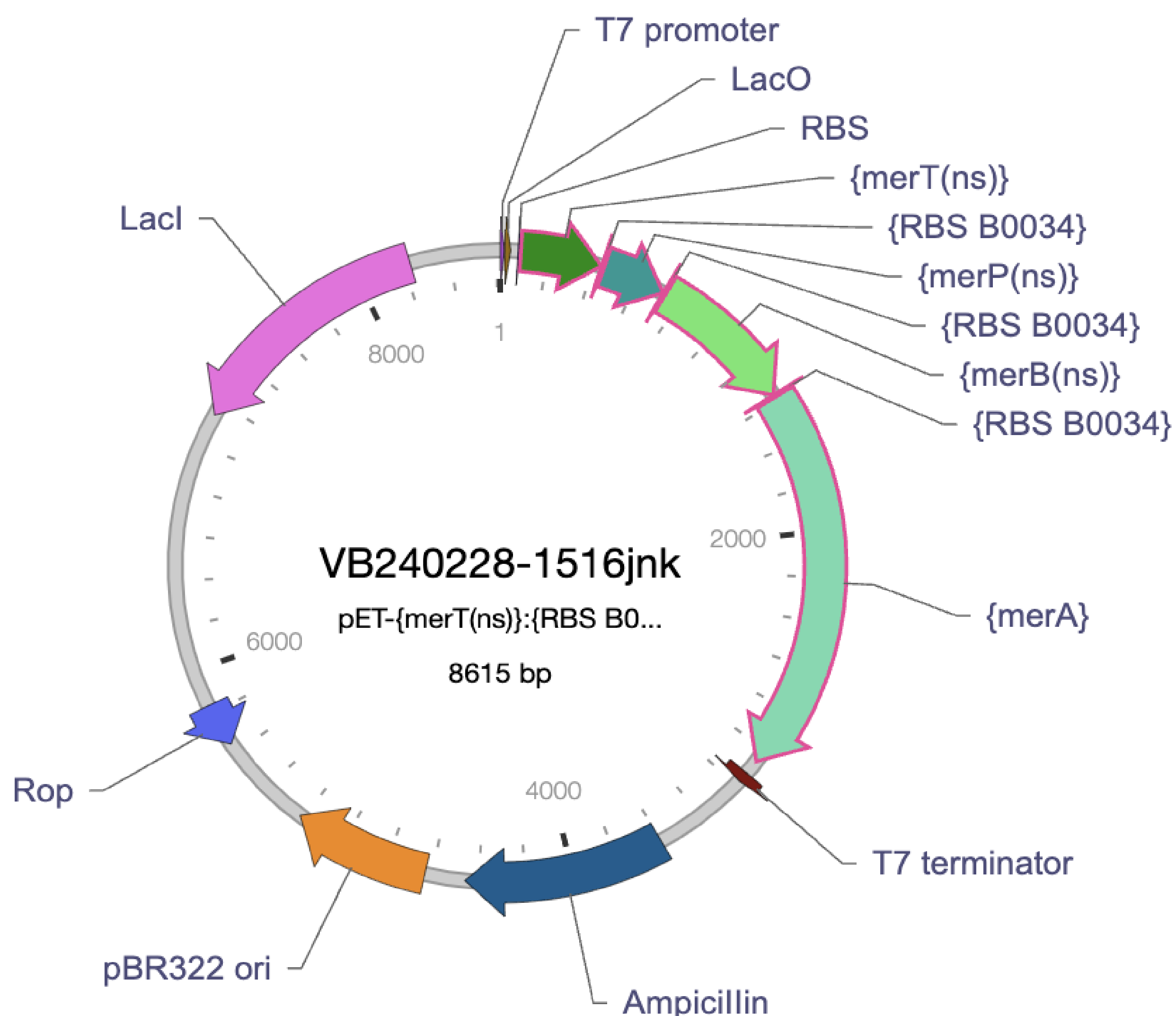

Figure 7. This is an image of our plasmid with the genes of interest, the ribosomal binding sites, and the promoter/terminators.

stay in the fish and affect human consumption. Currently, the canning process includes a step in which the fish is steamed prior to being separated into separate parts. The fish will be steamed at 165 °C as seen in Figure 3 and the *E. coli* plasmid dies at just 70 °C (World Health Organization, 2018). Not only that, because fish are warm-blooded animals, *E. coli* strains tend to slightly increase and then plummet down to no *E. coli*, which is needed to ensure human safety (Del Rio Rodriguez, 1997). The cooking process guarantees that the *E. coli* won't be affecting any biological food chains or ecosystems because it will all be removed after the steaming process. This ensures the containment of this experiment, and if any accidents or failures arise from experiments it won't affect a large population as a whole. Integrating with the current canning system will save energy and resources and also allows us to not include a kill switch in the plasmid, allowing us to shorten the length of the plasmid and include other parts.

To ensure that this idea works safely, and that mercury is properly removed, we would like to try our systems to test the penetration and effect of the bacteria. This can reveal any possible problems with our plasmid or the method of detoxification which results in no

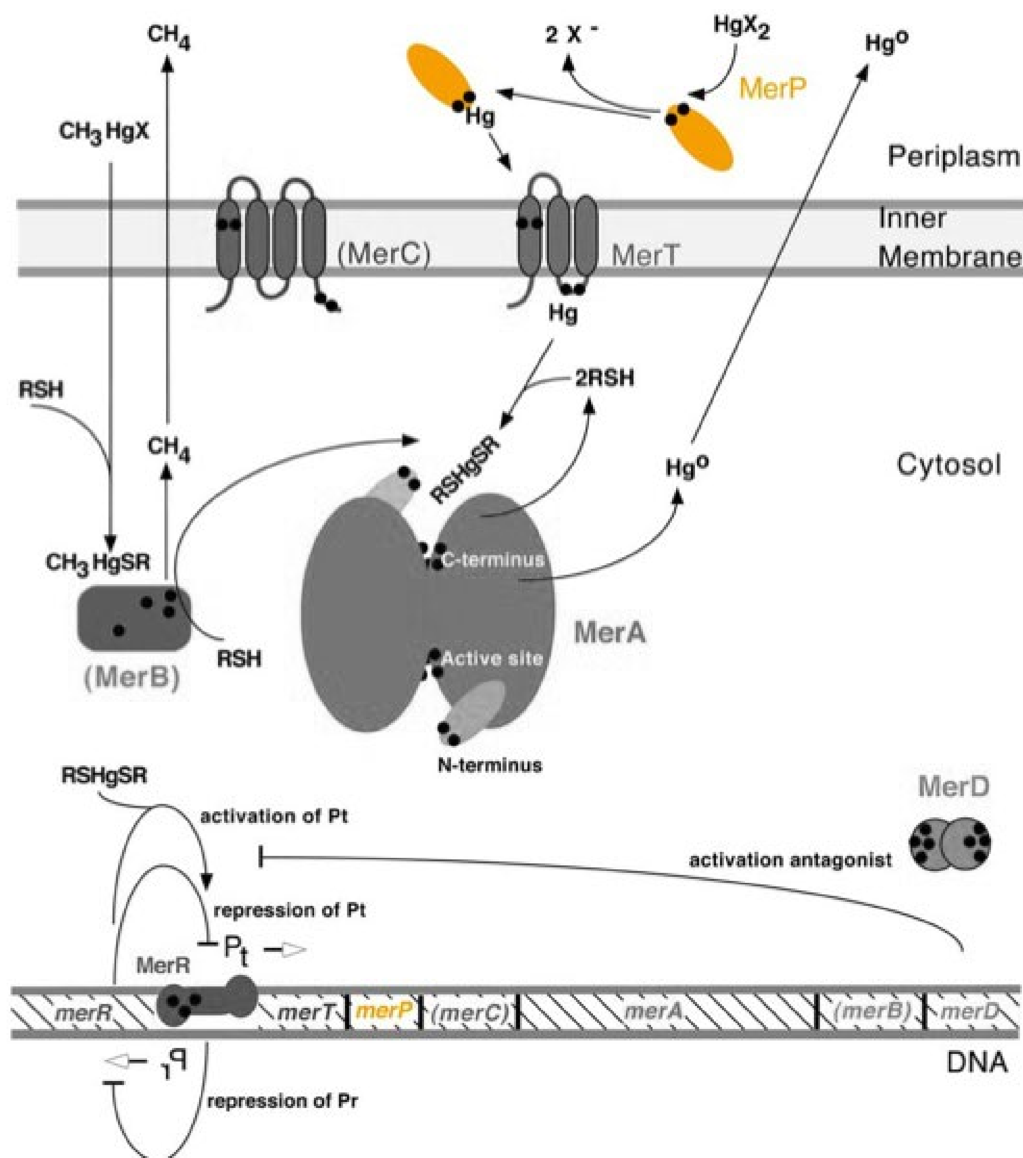

Figure 8. This image shows the process of the Mer protein interactions and how they work together to transport the mercury and breakdown the methylmercury into elemental mercury as a system.

net gain in the sense of mercury. While mercury is dangerous to humans, because all forms of mercury are contained within the fish along with the process of breaking down methylmercury, we would not be exposed to mercury in even trace amounts. However, we would still take general lab safety precautions when running any labs such as gloves, goggles, etc. We will also not need to worry about handling mercury ourselves because almost all fish have mercury in them, so we will not need to manually put the mercury in the fish. However, to confirm that the fish do have mercury inside of them, we can use gas chromatography (GC) coupled with inductively coupled plasma mass spectrometry (GC-ICP-MS) to precisely measure the mercury levels in the fish.

Another safety concern we have with the idea is that methane is produced as a byproduct of the MerB protein, as seen in Figure 4. While methane tends to be less dangerous than methylmercury, methane intake by humans can lead to harmful symptoms such as mood changes, slurred speech, and memory loss, and in extremely

high concentrations, methane consumption can lead to breathing impairment and abnormal heart rates (Public Health England, 2019). In order to prevent these harmful effects, we would like to conduct experiments to show that the methane produced is negligible enough to have no effect at all on human health, or we need to eliminate the methane before the fish are sold. One possible area we would like to explore is possibly using methanotrophs, or prokaryotes that use methane as their main source of energy, to remove the methane created as a byproduct (Hanson et al, 1996). We could possibly find a gene extracted from a methanotroph that breaks down methane and create a separate plasmid using the gene, and research at what times it would be best to utilize the plasmid, such as after the majority of the production of methane after the use of the Mer proteins.

The problem with using *E. coli* as the chassis for the plasmid would be the release of endotoxins into the fish when the cell membrane breaks down. This can be harmful to humans and cause a lot of respiratory problems when eaten in large quantities. The breaking down of endotoxins, however, doesn't require a huge change in the process because as long as another heating stage is set for the fish after the submersion the endotoxins would be removed. The specificity of this heating stage would be that it has to be dry heating at 250 ºC for 30 minutes (Batista et al 2007). This will remove a very high majority of the endotoxins in the body which will allow for quick removal and won't delay the canning process majorly. If incorporated alongside the breaking down of the *E. coli* process mentioned above, the canning of the fish would not require a big equipment change to be implemented into canning factories. All the above problems and solutions are already mostly incorporated into the canning of the food, so much of the safety problems going into the canning of the fish are problems that owners of canning factories have to be aware of rather than cautious of.

Discussions

Mercury contamination in fish poses a significant threat to human health. This research project attempts to solve this problem by proposing an approach for detoxification using genetically modified *E. coli* bacteria. We have designed a specialized plasmid that equips *E. coli* with proteins to detoxify the mercury out of the fish. This plasmid contains genes for four key proteins: MerT, MerP, MerA, and MerB. The first two, MerT and MerP, act as a tag team, transporting methylmercury, the most common form found in fish, into the *E. coli* cells. Once inside because of MerT and MerP, MerA and MerB take over, converting the harmful methylmercury into a much less toxic form, elemental mercury.

This approach has several advantages. *E. coli*'s rapid reproduction allows for large-scale detoxification, making it a practical solution. Additionally, the method integrates with existing fish canning procedures. Since canning involves heat treatment, it also eliminates the introduced *E. coli*, ensuring food safety for consumers. However, certain challenges need to be addressed before widespread adoption. The effectiveness of the method hinges on the *E. coli*'s ability to penetrate deep enough into the fish tissue to reach the mercury. Furthermore, the process generates methane gas as a byproduct, necessitating further research to minimize or eliminate its production. The size of the plasmid itself might also pose a limitation, potentially restricting the addition of genes for methane removal. As mentioned before, the use of our system would result in a reduction of 97% over a period of 10 hours, and we would conduct further experiments to see at what time the system reaches near 100% reduction.

Next steps

We have located a part (BBa_K3470017) that contains the GFP molecule. It can be attached to our current promoter sequence, so when we test for penetration, we will be able to tell the effects. This promoter sequence also is able to delete MerA and MerB to show changes between different test groups. We can create multiple groups to show the effectiveness of the Mer proteins by measuring the amount of methylmercury and

nonvolatile mercury in the plasmid with the MerA and MerB present, one where only MerB is deleted, a control with MerA and MerB are deleted, and other variations of our plasmid.

We would like to try our proof of concept to test the penetration and effect of the bacteria. The first step would be to have three bacteria cultures. We would like to transform one of the bacteria cultures to contain *E. coli* with the plasmid containing part BBa_K2123202 with an attached GFP molecule, one with the plasmid containing part BBa_K3470017, and one *E. coli* with no changes. We would then prepare fish samples containing methylmercury at different depths into the skin, place the fish into each of the *E. coli* solutions, and check for the presence of methylmercury in each of the fish. Then we would record our data to see what level the penetration stops at. We would do this by using gas chromatography (GC) coupled with inductively coupled plasma mass spectrometry (GC-ICP-MS) to measure the amount of methylmercury before and after.

We would also like to work with and contact engineering specialists that could help us design a suspension that will be able to interact with the plasmid and the fish. The suspension will allow for the fish to be carried out and submerged in different batches without having to remove the *E. coli* solution in each one. The suspension, however, would also have to not block any fish or parts of the fish from undergoing the detoxification.

From here, we want to contact companies that make canned fish and talk to them about how we can implement this idea and the added step. We would like to work with them on how to use a large enough suspension machine that is able to run for long enough for the detoxification benefits.

Another next step that we would want to go through is eliminating the methane byproduct. The concentration of the methane produced is negligible, meaning that it is not a large issue, but removing it would be the best course of action. However, the size of the plasmid may limit us from adding another component to take out the methane.

Author contributions

The contributors to the abstract were A.M., D.R., D.P., K.A., and K.B. The contributors to the video were D.R., D.P. and A.M. The contributors to the background were D.R., A.M., K.B., and D.P. The contributors to the systems level were A.M., D.P., and D.R. The contributors to the device level were A.M. and D.R. The contributors to the parts level were A.M. and D.R. The contributors to the safety section were A.M. and D.P. The contributor to the discussion was A.M. The contributors to the next steps were D.R., D.P., and A.M.

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