Improving stain removal using oleate hydratase-based solutions

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As many common laundry detergents are not biodegradable and can contaminate the environment with toxic chemicals, the purpose of our project is to develop an enzyme-based process that can degrade laundry stains more effectively and sustainably than current cleaning agents. Oleic acid is a common fatty acid in vegetable oils, notably olive oil, canola oil, and soybean oil. The project aims to produce an enzyme (oleate hydratase) that can be used in detergents to make oleic acid more soluble and essentially minimize fabric stains. By increasing the effectiveness of the detergent, less would be needed, and there won't be as much damage done to the environment. Oleate hydratase can be used to hydrate oleic acid, forming 10-hydroxystearic acid. When compared to oleic acid, 10-hydroxystearic acid has an additional hydroxyl group, potentially increasing the polarity and thus the solubility of the molecule. Then a recombinant plasmid will be created with a gene that encodes for oleate hydratase from *Elizabethkingia meningoseptica*. Afterwards, *Escherichia coli* will be used to create oleate hydratase enzymes to degrade oleic acid. We plan to use transformation to insert our recombinant plasmid. By transforming the gene coding for oleate hydratase into *Escherichia coli* bacteria, oleate hydratase proteins can be created. We will then purify the proteins from the bacteria, first by lysing the bacteria, centrifuging the solution, and then isolating the proteins from the supernatant through nickel column chromatography. he stain removal can be further improved by changing other variables. It was hard to determine what genes and what plasmid we would use, but we did not come across many challenges. This is likely because we were unable to implement our design to see what worked and what didn't. Afterwards, the purified enzyme's ability to hydrate and remove notoriously difficult lipid stains on fabrics with oleic acid can be observed and quantified by measuring solubility to show the possibility of improving stain removal.

Keywords Oleate hydratase, stain, detergent, fabric, *Escherichia coli*

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Watch a video introduction by the authors at https://youtu.be/f4BesQC0pJk

Background

One important step towards becoming more environmentally conscious is addressing the harm that laundry detergents have on aquatic ecosystems. Many common laundry detergents are not biodegradable (Mitra, 2012) and can contaminate the environment with toxic chemicals, such as how phosphates in the detergent may collect in waterways and cause eutrophication, or excess nutrients in aquatic ecosystems, causing a steep increase in vegetation growth and killing organisms as a result of a decrease in oxygen levels (Hill, 2018). Over 30 billion loads of laundry are washed per year, each using about 40 grams of toxin-filled detergents (McKenzie). Some laundry detergents even contain elements that have been classified as carcinogens by the Environmental Protection Agency (McKenzie). The purpose of our project is to develop an enzyme-based process that can degrade laundry stains more effectively and sustainably than current cleaning agents. The primary components of laundry detergents generally consist of water softeners, which remove soap scum-forming metal cations from the water; bleach, which targets certain organic stains; surfactants, which allow soils to be absorbed and emulsified; and enzymes, which break down specific biomolecules (Smulders 2007). The detergent chemically interacts with the stain, causing the molecules of the stain to move out from between the fabric fibers. Once the stain has moved to the surface of the fabric, it can be washed away with water. Many existing laundry detergents contain phosphates, which promote algae blooms that starve other aquatic life of oxygen (Beach 2017). Another ingredient common in detergents, surfactants, can break down the mucus layer of fish, leaving them vulnerable to parasites and bacteria. Additionally, surfactants reduce the surface tension of water, leaving waterways more susceptible to absorbing pollutants and pesticides, and break down into more toxic waste. By contrast, enzyme washes easily break down in the environment as organic waste (Krosofsky 2020). Common enzymes include proteases, lipases, amylases, and cellulases. Oleic acid is a commonly found fatty acid in vegetable oils like olive oil, canola oil and soybean oil. Oleic acids are the most common and widespread natural fatty acid, so by targeting oleic acid, the solubility and degradation of lipid stains can be increased (National Center for Biotechnology Information). The project aims to produce an enzyme, oleate hydratase, that can be used in detergents to make oleic acid more soluble and make the detergent more effective so that less of it is needed. As a result of using less detergent, there would be less damage done to the environment. Oleate hydratase can be used to hydrate oleic acid, forming 10-hydroxystearic acid (Engleder et al., 2015), which contains one more hydroxyl group, potentially increasing the polarity and thus the solubility of the molecule. A recombinant plasmid can be created with a gene that encodes for oleate hydratase from *Elizabethkingia meningoseptica*, and then *Escherichia coli* can be used to create oleate hydratase enzymes to degrade the oleic acid. In our design, we use transformation to insert our recombinant plasmid. By transforming the gene coding for oleate hydratase into *E. coli* bacteria, it can create oleate hydratase proteins. We will then purify the proteins from the bacteria. Afterwards, its effectiveness at hydrating and removing notoriously difficult lipid stains on fabrics with oleic acid can be observed and quantified by measuring solubility.

Systems Level

The production of oleate hydratase is planned to take place once the DNA sequence from *E. meningoseptica* that codes for oleate hydratase is inserted into *E. coli* in a recombinant plasmid. Once the oleate hydratase is produced, the enzyme will be purified and extracted.

The oleate hydratase will then be used for laundry stains composed of oleic acid containing lipids. Once the enzyme solution is added, oleate hydratase will catalyze the hydration of oleic acid into 10-hydroxystearic acid (BRENDA 2021). This new compound has more hydroxyl groups compared to oleic acid, thus making it more water soluble.

Device Level

Dirt and other molecules hard to wash from clothing are often nonpolar molecules, which bond with water less strongly than inter-water hydrogen bonding, leading to lower solubility and ability to be washed away. *E. coli* are engineered to produce the enzyme oleate hydratase after being transformed via a recombinant plasmid with genes coding for the enzyme taken from *E. meningoseptica*. The functional enzyme adds a hydroxyl functional group to oleic acid, a common fatty acid found in oil-based stains. The highly electronegative oxygen in the hydroxyl functional group allows for the group to form hydrogen bonds with water, allowing the molecule to bond with water more strongly, making it more soluble and more easily washed away.

Parts Level

The ohyA gene from *E. meningoseptica* is modified with the addition of a His6 amino acid tag to better facilitate protein purification (Engleder et al., 2015). This gene is placed into the pET-28 plasmid; the plasmid can be created with restriction enzymes (Engleder et al., 2015). It can be transformed into the One Shot® BL21 Star™ (DE3) Chemically Competent *E. coli* bacteria. These restriction enzymes both create sticky ends to attach the new DNA to the sequence and create blunt ends to

cut out unused DNA. The T7 promoter for the plasmid containing the genetic sequence for oleate hydratase and other components are left in the *E. coli* bacteria to allow for the creation of the oleate hydratase protein.

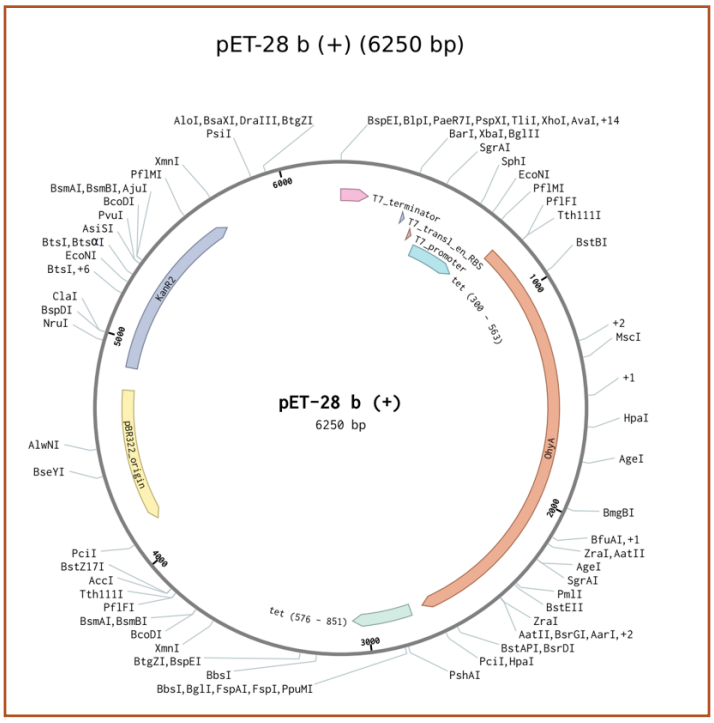

Figure 1. *Modified pET–28 b(+) to contain optimized ohyA gene sequence for expression for His6-OhyA in E. coli. Figure created by Benchling software.*

The selectable marker and the origin of replication were maintained in the plasmid.

Safety

This research will be conducted in a clean and safe lab using sterile and clean equipment. The surfaces the experiment will be conducted on will be thoroughly sanitized with bleach or another cleaner before and after the experiment. The bacterial DNA from *E. meningoseptica* will be used to create a plasmid for oleate hydratase, after which *Escherichia coli* will safely be used to create the oleate hydratase enzymes to degrade the oleic acid on the clothing. The One Shot® BL21 Star™ (DE3) Chemically Competent E. coli strain being used in the experiment is biosafety level 1 and isn't harmful to humans. Solutions that have been contaminated will be disposed of and the containers they were in will be sanitized with a 70% ethanol or a 10% bleach solution and RNAse (McDonnell & Russell, 1999). After the experiment is completed, the experiment's findings will be analyzed to see how it can be improved to better degrade the stain. Additionally, participants will wear gloves and safety goggles throughout the experiment/testing and will thoroughly wash their hands before leaving the lab area.

Discussions

However, the effects of transforming oleic acid into 10-hydroxystearic on the detergent's overall ability to degrade stains still needs investigating. Since oleic acid is only one potential component of a stain, its increased solubility may not have a significant impact on the common stains encountered in laundry. Additionally, in our research project, we decided to use a single enzyme for the detergent. Upon doing research on the enzyme, oleate hydratase, we predict that the quality of this enzyme will vary after it gets purified from *E. coli*. More research is needed to include a more diverse spectrum of enzymes and explore their different characteristics and abilities to degrade common stains. We also plan to research how we can purify enzymes without changing their effectiveness. Overall, with more time and research, we can discover more enzymes that are able to degrade stains with maximum efficiency. We ran into some challenges when finding what enzyme and materials to use, although we did not encounter many benefits and obstacles because we were unable to implement our design. When finding the enzyme, we needed to make sure it would not damage the fabric, while also effectively removing stains and other functions it would need to perform. We had to overcome these types of obstacles to ensure that our design would work. One challenge in our project design is determining how much more environmentally friendly our detergent is compared to others, and whether it will make a significant impact when used in real life.

Next Steps

In the future, potential next steps could include determining what environment conditions could be changed to increase the effectiveness of oleate hydratase, such as pH and concentration. We could then design our detergent to recreate those conditions to maximize oleate hydratase's ability to degrade stains.

We plan to also explore the option of creating the detergent solely of enzymes to enhance the range of stains this detergent can remove. Currently, there are no detergents made of only enzymes. This should be researched because it could help determine what should be added to the detergent design to increase its effectiveness. Experiments that determine the effect of pretreating the fabric with other enzymes on oleate hydratase's performance, by measuring the amount of stain removed, could be performed. The amount of hydratase, types of stains and fabrics, and the duration of time that the hydratase stays on the fabric would

be the controls for the experiment and the types of enzymes added for pretreatment would change. This would help determine if oleate hydratase is more effective at removing stains in the presence of another enzyme and if so, which enzyme.

Author Contributions

Idea generation: A.H., A.L., S.L., A.M., M.M., N.M., W.M., E.T.S., J.W., and J.Y. generated the project idea. A.H., A.L., S.L., A.M., M.M., N.M., W.M., E.T.S., J.W., and J.Y. carried out research on the topic. A.H., A.L., S.L., A.M., M.M., N.M., W.M., E.T.S., J.W., and J.Y. contributed to the project design. E.T.S. wrote the background section of the manuscript and video, A.L. wrote part of the systems-level section of the manuscript and video, J.Y. wrote part of the systems-level section of the manuscript and video, N.M. wrote the device level section of the manuscript and video, W.M. wrote the part level section of the manuscript and video, S.L. wrote part of the safety section of the manuscript and video, J.W. wrote part of the safety section of the manuscript and video, A.H. wrote part of the discussion section of the manuscript and video, A.M. wrote part of the discussion section of the manuscript and video, and M.M. wrote the next steps section of the manuscript and video.

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