Design Brief

PET-degrading cyanobacteria for marine environments

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A lesser known category of plastic pollution is microplastics: pieces of plastic less than 5 mm in diameter. Microplastics carry a multitude of negative impacts, notably, the bioaccumulation in marine organisms, which harms marine resources. As the largest archipelago in the world, Indonesia has become the 8th most marine resource dependent country. This inspired the goal of the design: to break down polyethylene terephthalate (PET) microplastics for the preservation of the marine environment. Due to its key contributions to atmospheric oxygen, the marine organism cyanobacteria was the chosen chassis. The design involves the PETase and MHETase gene found in Ideonella sakaiensis, which breaks down PET into terephthalic acid (TPA) and ethylene glycol (EG). To allow the gram-negative cyanobacteria to secrete the enzymes, a type 2 secretion system (T2SS), twin arginine translocation pathway (Tat) and signal gene from Escherichia coli were incorporated. A sfGFP gene was also added to determine whether the cyanobacteria has been successfully transformed. Lastly, all of the aforementioned genes were located downstream of the NahR inducer and pSal promoter, which only allows for transcription to occur in the presence of salicylate acid. All these genes were then inserted into the pBAD LIC cloning vector 8A, an empty plasmid backbone. Though the costs, regulation of enzyme production and impact of byproducts were considered, there are still several limitations. These limitations are the number of base pairs in the plasmid sequence, the assumption that the enzymes would function in marine conditions and the reliance on the untested pSal-NahR promoter.

Keywords: PETase, cyanobacteria, marine, PET Degradation

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Watch a video introduction by the authors at https://youtu.be/hu_lyClt4qI

Background

Plastic pollution

What are plastics?

Plastic is a synthetic material that is made from organic compounds. These compounds are normally extracted from natural gas, but can also be from petroleum. The raw natural gas or petroleum would be processed through cracking and dehydrogenation to create unsaturated hydrocarbons in the monomer form. When the monomers bind to create a chain of repeating units, a high molecular mass structure, known as a polymer, forms. (Wilson and Killinger 2020)

Common household plastics would include polyethylene (PE), polyvinyl chlorides (PVC) and polyethylene terephthalates (PET). PET consists of benzene rings, oxygen and nylons which are incorporated with nitrogen and hydrocarbons (Brunning 2015). Most household plastics are hydrophobic due to their non-polar nature, where the electron charges are evenly distributed in the molecule.

Plastic production has expanded exponentially, from 2.3 million tons in 1950 to 448 million tons by 2015. In fact, eight million tons of plastic waste enters the sea from waterfronts (Parker 2020). A large portion of plastic waste is in the marine environment since most water bodies lead to the ocean. Once adrift, plastic garbage remains in beachfront waters, and may travel around the world to end up in the Great Pacific Garbage Patch, a gyre of marine debris. This particular gyre is estimated to have a massive amount of 1.1 to 3.6 trillion pieces of plastic (McGregor n.d.).

What is polyethylene terephthalate?

Polyethylene terephthalate (PET) is made from the polymerization of ethylene glycol and terephthalic acid, organic compounds distilled from crude oil. The use of PET is widespread since it is not only durable, but also light-weight and waterproof. One of the most well known uses of PET is to contain beverages, however, it is also found in synthetic clothing and automotive parts. PET is non-biodegradable as most microorganisms are unable to break it down. Within a long period of time, plastics can naturally break down with the exposure of UV light through a natural photochemical process.

What are microplastics?

Microplastics are pieces of plastics with a diameter less than 5 mm in length. There are two types of microplastics, primary and secondary. Primary microplastics are pieces of plastics which are small in size that enter the environment directly from a variety of sources, such as toothpaste and exfoliators. In these products, the type of microplastics found are known as microbeads. When these microbeads are washed down the drainage system, they will eventually end up in the ocean since they easily pass through filters. Another example of primary microplastics are plastic pellets, which are melted to form plastic products. These pellets, when transported from one factory to another, may spill out of the transportation vehicle and be blown away into a water body. Secondary microplastics come from larger pieces of plastics, such as fishing nets and bottles, that have undergone weathering. In the marine environment, the weathering is caused by ocean waves, wind abrasion and ultraviolet radiation.

Why are microplastics dangerous?

Microplastics are not biodegradable, therefore they remain in the marine environment for 450 to 1000 years. During this time, microplastics pose numerous threats to marine life. When ingested, microplastics accumulate and obstruct the digestive systems. This leads to starvation, or even worse, internal bleeding when an organ is punctured. To make matters worse, microplastics adsorb chemical pollutants, which also enter the digestive system. Both the chemical pollutants and microplastics undergo biomagnification, a process where pollutants increase in concentration as trophic levels increase. As of result, populations of fish would diminish over time with the accumulation of microplastics in the ecosystem.

Though microplastics itself have not been proven to harm humans, they are being consumed more often than you would expect. According to a study conducted in 2018 by Mason and her team, 93% of the 259 bottled water samples tested contained traces of microplastics. Mason investigated 11 of the most popular bottled water brands, with Nestle Pure Life having the highest average concentration of microplastics (Mason, Welch and Neratko 2018). Multiple studies have also shown the presence of microplastics in table salt. A study conducted by Korean researchers along with GreenPeace East Asia concluded that microplastics are found in 90% of table salt (Kim, Lee, Kim, et al. 2018). The list does not end here. Humans ingest microplastics when consuming seafood, especially shellfish. The average European shellfish consumer is said to ingest up to 11,000 microplastic particles per year.

In the near future, plastic pollution promises to be detrimental towards many economies around the world, especially Indonesia, the world’s largest archipelago. Indonesia has endless amounts of coastlines that sustain and support the numerous communities that inhabit these areas which depend on them for their livelihoods.

People of Indonesia, such as fishermen and dive guides, use these coastal areas for a variety of livelihoods that rely on marine resources for economic sustainability. The standard of living of these communities are at risk as plastic continues to threaten the availability of marine resources. The fishing and tourism industry both rely on marine resources, which contributed towards 6.64% of Indonesia's national economy in 2010 (Nurkolis, Nuryadin, Syaifudin, et al. 2016).

Ideonella sakaiensis

In 2016, Japanese researchers discovered Ideonella sakaiensis, a bacterium which breaks down polyethylene terephthalate (PET) (Andersen 2019). The enzymes found in I. sakaiensis, known as PETase and MHETase, cooperate to depolymerise PET into the monomers of ethylene glycol (EG) and terephthalic acid (TPA). These final products are a major energy and carbon source for I. sakaiensis to grow.

Cyanobacteria

What are cyanobacteria?

Cyanobacteria, also referred to as algae, are bacteria that use and produce energy from oxygenic photosynthesis, a process that involves chlorophyll. These bacteria can be found in aquatic habitats, such as fresh, brackish and marine waters, but have also been found in extreme environments, like hot springs, cold lakes and deserts. Cyanobacteria are thought to be responsible for triggering the "Great Oxygenation Event", which drastically altered Earth's atmosphere by increasing its oxygen concentration.

Cyanobacteria is abundant in the marine environment and relatively easy to culture in a lab environment. In addition, their ability to carry out nitrogen-fixation and carry out photosynthesis makes them self-sufficient for energy. Hence, cyanobacteria is an ideal chassis for this design.

How does cyanobacteria photosynthesise?

Energy is released by cyanobacteria in the process of oxygenic photosynthesis. This is the process where water is used as an electron donor in order to produce oxygen (Frain, Gangl, Jones, Zedler, et al. 2016), unlike anoxygenic photosynthesis, which does not produce oxygen (Photosynthesis in Bacteria). Cyanobacteria do not contain chloroplasts. Rather, they contain chlorophyll, mostly chlorophyll a (Chl a), in thylakoids. Chl a absorbs blue-violet and orange-red light to convert it into chemical energy that will be used for metabolic processes ("What Are The Roles Of Chlorophyll A & B?").

What is the importance of cyanobacteria?

Cyanobacteria fossils have been on this planet for at least three billion years, and have contributed to the current 21% of oxygen that we respire upon today. In the Archaean and Proterozoic period, a vast quantity of oxygen was produced by cyanobacteria, which was absent before this evolution. Prior to the evolution of cyanobacteria, life forms were anaerobic and no free oxygen was present in the atmosphere due to its reactivity (Biello 2009). It is suspected that cyanobacteria gained the ability to photosynthesise through horizontal gene transfer (HGT). These genes were then passed down from one progeny to the next, which provided a higher survival chance since energy could be derived from the sun. The widespread ability to photosynthesise created large amounts of oxygen that became largely incorporated into the atmosphere. To this day, all photosynthetic organisms can be traced back to this single evolution where such organisms, at some point in their own evolution, included cyanobacteria into their cells (Soo, Hemp, Parks, et al. 2017). This process has eliminated the existence of many anaerobic organisms, and instead gave rise to oxygen-dependent organisms.

What is the structure of cyanobacteria?

Unique to cyanobacteria are the organelles responsible for the photosynthetic machinery known as thylakoids, which are flattened membrane vesicles that contain chlorophyll. Cyanobacteria are gram-negative organisms, meaning that they possess both inner and outer membranes and a thin layer of peptidoglycan located between them. The double membrane creates periplasmic space, also known as the periplasm that separates the two layers (Donnenberg 2015). It contains high concentrations of peptidoglycan alongside proteins (including proteases and phosphatases) and is filled with periplasmic liquid, forming an aqueous environment which allows for oxidation (Periplasm 2020). In addition to this, the gram-negative bacteria is less permeable in comparison to gram-positive bacteria (Fischer and Braun 1981). This is because there are more layers which act as barriers in gram-negative bacteria than in gram-positive bacteria, which have a simpler structure (Singh, Young and Silver 2017).

What is Synechococcus?

Synechococcus is a genus of cyanobacteria that is regarded to be the most well-studied, therefore making it the most appealing choice for this project. Synechoccus can be found in all marine environments, except several areas in Antarctica, in the euphotic zone; the uppermost layer in the ocean. Sunlight is especially

essential to Synechoccus since it is solely phototrophic, unlike many other cyanobacteria.

Systems level

The system aims to secrete PETase and MHETase enzymes through the twin-arginine translocation pathway (Tat) and the type two secretion system (T2SS), but only in the presence of PET).

Despite being less well-known, cyanobacteria were the chosen chassis since it would allow for the net production of carbon dioxide as any carbon dioxide produced will be used by the cyanobacteria for photosynthesis. Carbon dioxide can be produced from the degradation of TPA. When TPA is broken down, it will produce succinate, which would enter the tricarboxylic cycle. In this cycle, the succinate will be broken down into ATP and carbon dioxide.

Being extremely miniscule, cyanobacteria cannot carry out phagocytosis, a process in which materials are engulfed by a cell by using its membrane. Instead, a process called 'pomacytosis' is carried out, where the material to be engulfed is held in the mouth of the cell membrane. Pinocytosis is another process, which utilizes the cell membrane, although it should be noted that both 'pomacytosis' and phagocytosis differ from it. In pinocytosis, liquids, not solid material, are engulfed. As such, the initial idea was for the microplastic to be taken into the cyanobacteria through 'pomacytosis', however, this would be impossible as the microplastic is too large (Kamennaya, Kennaway, Fuchs, et al. 2018). Due to this, the idea of secreting the enzymes was chosen instead. PETase consists of four chains, which are held together by four disulfide bonds, whereas MHETase has an unknown number of chains held together by six disulfide bonds. The nature of these enzymes require channels that transport folded proteins, and thus the Tat and T2SS was chosen. Additionally, these two channels are able to transport the proteins through the gram-negative cell membrane.

To regulate enzyme production, the NahR-pSal gene has been chosen. Salicylate acid, the chemical byproduct in the natural degradation of PET, binds to the NahR gene, which induces the pSal promoter. This begins the synthesis of the proteins downstream. It is important to note that the PET does not induce the NahR gene. Unnecessary production of the enzymes will consume energy, reducing the survival of the cyanobacteria, and it would also harm the environment as these enzymes are foreign proteins. Therefore, the regulation of enzyme production is crucial.

Device Level

The designed plasmid will synthesise PETase, MHETase and superfolder green fluorescent protein (sfGFP) in the presence of salicylate acid that forms on the surface of microplastics. This would allow for the breakdown of PET microplastics only when it is present as the expression of the genes are regulated by the NahR-pSal promoter (Figure 1).

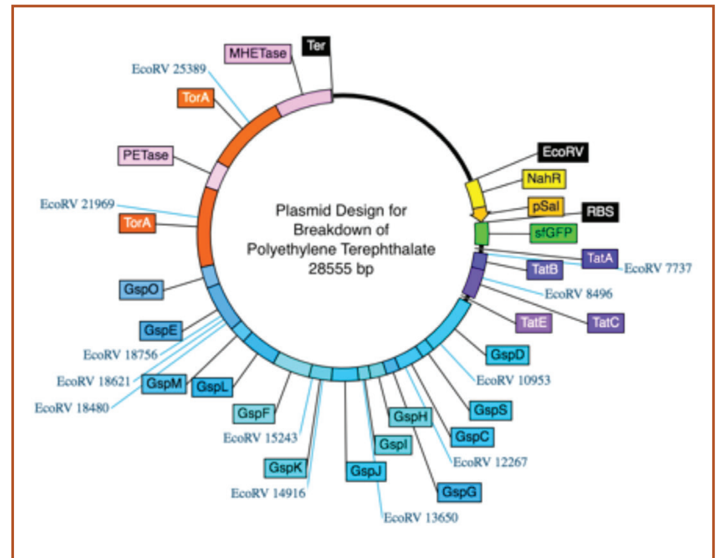

Figure 1. Plasmid design for the breakdown of PET.

Parts level

NahR-pSal

The NahR gene and pSal promoter were found in the NAH7 plasmid in Pseudomonas putida (Anderson 2007). This plasmid encodes for enzymes that break down naphthalene into salicylate, pyruvate and intermediates of the tricarboxylic (TCA) cycle. Therefore, naphthalene is a carbon and energy source for Pseudomonas putida as the intermediates will undergo chemical reactions to release ATP and carbon dioxide. However, in this design, only the salicylate detecting genes were included, because naphthalene is not an intermediary compound of PET degradation.

The NahR-pSal promoter operates on the basis of structural changes caused by the presence of salicylate. The NahR is binded to the pSal region on the DNA, forming what is known as the NahR-DNA complex. However, when salicylate acid is present, this complex undergoes a structural change, which activates the hydrolysis of ATP and the opening of the DNA for

transcription. Therefore, including this in the design would ensure that proteins are only synthesised in the presence of salicylate, the chemical released in the natural degradation of PET.

Superfolder Green Fluorescent Protein

Superfolder green fluorescent protein (sfGFP) is a protein derived from Aequorea victoria, a bioluminescent jellyfish (Lambert 2020). To be specific, it is a weak dimer; consisting of two monomers that have weak intermolecular forces . In this design, the sfGFP would allow for the identification of cyanobacteria that have been successfully transformed. When sfGFPs are present, it indicates that the designed plasmid has been taken up by the cyanobacteria. Furthermore, if the sfGFP is only seen when there is PET present, it would indicate that the NahR-pSal promoter is functioning correctly. To check for the presence of sfGFP, the culture of cyanobacteria should be placed under a UV light.

Twin Arginine Translocation Pathway

Generally, twin arginine translocation (Tat) pathways transport folded proteins through the inner membrane of a cell into the periplasm. Since cyanobacteria is gram-negative, the genes for the Tat pathway were essential as it would allow for the secretion of enzymes. In this design, the genes, TatABCE, were taken from E. coli strain K12.

Type 2 Secretion System

Similar to the Tat pathway, the type 2 secretion system (T2SS) also transports folded proteins, but from the periplasm through the outer membrane instead. The 13 genes, GspGHIJK, GspD, GspS, GspC, GspFLM, GspO, GspE, for the T2SS were also taken from E. coli strain K12. Along with the Tat pathway, the enzymes would be able to travel from inside the cell, through the inner membrane and outer member, to exit the cell for secretion.

TorA Signal Protein

TorA, an abbreviation for trimethylamine-N-oxide reductase 1, has an N-terminal signal peptide sequence that is crucial to secrete PETase and MHETase. By fusing the signal peptide sequence to the PETase and MHETase enzymes, the Tat pathway will recognise it and be able to secrete it. It is important to note that the full TorA protein itself does not need to be expressed to transport the enzymes. At the Tat pathway, the AxA (ATA) motif at the C-terminal will be cleaved off by leader peptidases (Figure 2).

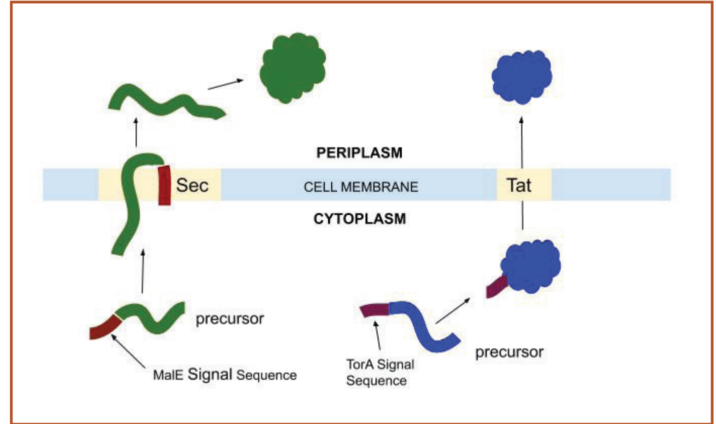

Figure 2. *The Sec and Tat Secretion Pathways.*

PETase & MHETase

Poly(ethylene terephthalate) hydrolase (PETase) is an enzyme that catalyses the hydrolysis of PET into an intermediary compound known as mono(2-hydroxyethyl) terephthalate (MHET), which undergoes hydrolysis to produce EG and TPA. This hydrolysis process is catalysed by Mono(2-hydroxyethyl) terephthalate hydrolase, or MHETase for short. Besides I. sakaiensis, the PETase gene is also found in Streptomyces sp. SM14 (Almeida, Rincón, Jackson, et al.2019), a marine derived strain, and Galleria mellonella (Bombelli, Howe and Bertocchini 2017), a wax caterpillar, where the MHETase gene is also found. This design uses the PETase and MHETase genes found in I. sakaiensis since the genes in the other organisms have not been characterised.

EcoRV

The restriction enzyme, EcoRV, cuts DNA, it leaves no unpaired bases or overhangs, which gives rise to the process of blunt end cloning. Sticky end cloning would be difficult to use for this project since there are no restriction enzymes that give rise to desirable cut sites that would fit with the terminator and NahR genes.

Safety

The design is targeted to break down PET microplastics. However, it may also cause unprecedented damage. For example, the equipment needed in fishing and shipping industries would be broken down since the design would hypothetically react to any salicylate acid released by plastic on the surface. In addition to this, PETase, MHETase and the byproducts of plastic breakdown are proteins foreign to the marine environment such that it may grow to become a threat to marine organisms. Even so, despite the fact that TPA is on the hazardous chemicals list of the Environmental Protection Agency

(EPA), it remains non-toxic to aquatic organisms at concentrations of 15mg/l at 10°C (Terephthalic Acid (TPA) n.d.). Considering the ratio of plastics to the volume of saltwater, it would be highly unlikely for the TPA to disrupt the ecosystem.

In order to prevent the potential harmful effects during the construction of the design, the tests should be conducted in a lab with the simulation of marine environment conditions. The developers should also be cautious of their actions, especially when working with corrosive chemicals or flammable chemicals. Even if the design is created into a final product, there must be more steps taken to investigate its negative and positive impacts on the marine environment.

Discussion

Limitations

Though the design would theoretically break down PET microplastics, there are several limitations found in the design. To begin with, the design has not been tested, making it difficult to determine whether the assumptions made during the development process are correct. This undermines how realistic the design is. Therefore, to further develop the design, many tests should be conducted towards the formation of the final product.

A test needs to be conducted on the NahR-pSal promoter to determine whether it detects the presence of PET plastic and the extent of its detection. This can be done by inserting the NahR-pSal and sfGFP into an empty plasmid backbone, which would then be used to transform E. coli. After culturing the E. coli, it can be exposed to PET plastic which has salicylate acid on its surfaces. The expression of sfGFP will show if the detection of PET plastic is working the way intended.

In addition, an investigation should be carried out on the effectiveness of PETase and MHETase in marine conditions since it does not have optimal conditions for these enzymes. The optimal conditions for PETase and MHETase are pH 9 and 40°C, and pH 7 and 30°C respectively (UniProt 2020a,b). The pH of Indonesian coastal waters have been found to range from 7.65 to 7.75 in the Karimata Strait and between 7.88 to 7.94 in the Banda Sea, but pHs as high as 8.1 have been recorded off the coast of Central Java (Putri, Setiawan and Safitri 2015). Ocean temperatures have been found to range from 26 to 31 OC, depending on the year and location, in the waters off the coasts of Indonesia (Kusuma, Murdimanto, Aden, et al. 2017). Based on the data, the conditions of the Indonesian waters are ideal for the MHETase. However, this is not the case for PETase as it requires an alkali solution and a relatively high ocean temperature, therefore, suboptimal.

Besides the lack of tests, another limitation is the number of base pairs, 28555, in the plasmid design. This is a very large number for a single plasmid, and so it would be more realistic to split up plasmid design into two. To create two , the Tat pathway and T2SS genes should be separated from the PETase, MHETase and TorA signal protein genes, resulting in the design below. (Figure 3)

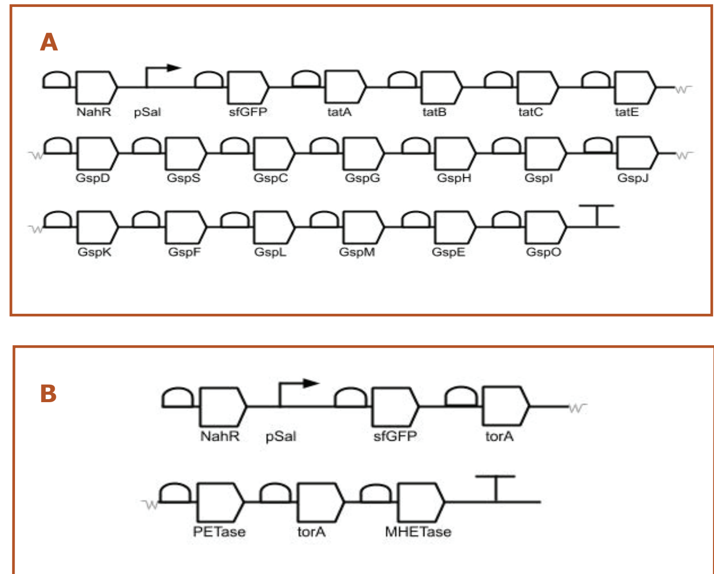

Figure 3. *Device 1 (A) and Device 2 (B) design revisions.*

Future Directions

Biofilm

Aside from mitigating the limitations, there are other ideas that can be pursued to improve the overall design. One of these ideas would be the utilization of biofilm, a glue-like, slimy substance that is secreted by microorganisms when they adhere to a surface during reproduction. If biofilm were used, it could possibly trap microplastics in it, therefore collecting it in one location and making it easier for the enzymes to break down the plastics. In order to do this, the NhaR gene could potentially be used since it heightens biofilm production.

Quorum sensing

Another idea is to implement quorum sensing, which would allow different cyanobacteria to communicate with one another. Through quorum sensing, gene expression is regulated in response to changes in the population density of cells. Hence, it would be ideal to cause cyanobacteria to release the chemicals used in quorum

sensing to cause an increase in population density when there is a high concentration of PET plastics.

Breakdown of TPA and EG

Though the PETase and MHETase is able to break down PET, the resulting products may still pose a threat towards the marine environment due to toxicity levels. Therefore, it would be a good idea to break down TPA and EG. TPA can be broken down by genes found in Comamonas sp. strain E6 into pyrrolidone carboxylic acid (PCA), which can be further broken down into succinate by genes from Acinetobacter baylyi. This would enable the succinate to enter the tricarboxylic acid cycle (TCA), hence allowing the cyanobacteria to break it down for ATP. On the other hand, EG would be broken down through a process of glycolysis by including native E. coli genes.

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References

Andersen M. Bacteria can break down plastic [Internet]. Denmark: Technical University of Denmark; 2019 Sep 30 [cited 2020 Apr]. Available from: https://www.dtu.dk/english/news/2019/09/eng-bakterier-har-faaet-appetit-paa-plast?id=afa835a7-eb08-4f05-a5ee-9212464dcf26

Anderson J. Part:Bba_J61051 [Internet]. Boston (MA): International Genetically Engineered Machine, Registry of Standard Biological Parts; 2007 Feb 20 [accessed 2020 Apr 24]. Available from: https://parts.igem.org/Part:BBa_J61051

Almeida EL, Rincón AC, Jackson SA and Dobson ADW. In silico screening and heterologous expression of a Polyethylene Terephthalate Hydrolase (PETase)-Like Enzyme (SM14est) with Polycaprolactone. Front Microbiol [Internet]. 2019 Oct 1 [cited 2020 Apr];10:2187. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6779837/

Biello D. The origin of oxygen in earth's atmosphere [Internet]. Springer Nature America Inc, Scientific American; 2009 Aug 19 [cited 2020 Apr]. Available from: https://www.scientificamerican.com/article/origin-of-oxygen-in-atmosphere/

Bombelli P, Howe CJ and Betocchini F. Polyethylene bio-degradation by caterpillars of the wax moth Galleria Mellonella. Current Biology [Internet]. 2017 Apr 24 [cited 2020 Apr];27(8):R292-3. Available from: https://www.sciencedirect.com/science/article/pii/S0960982217302312.

Brunning A. A guide to common household plastics [Internet]. Compound Interest; 2015 Apr 30 [cited 2020 Apr]. Available from: http://www.compoundchem.com/2015/04/30/plastics/

Cauwenberghe, L., & Janssen, C. (2014, July 05). Microplastics in bivalves cultured for human consumption. Retrieved October 18, 2020, from https://www.sciencedirect.com/science/article/pii/S0269749114002425

Donnenberg MS. Enterobacteriaceae. In: Bennet JE, Dolin R and Blaser MJ, editors. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. 8th ed. Vol. 2. (PA): Saunders; 2015. pp. 2503–17.

Fischer E and Braun V. [Permeability barrier of bacterial cell envelopes as cause of resistance to antibiotics (Author's Transl)]. Immun Infekt. [Internet]. 1981 [cited 2020 Apr];9(3):78-87. Available from: https://pubmed.ncbi.nlm.nih.gov/7019046/

Frain KM, Gangl D, Jones A, Zedler JAZ and Robinson C.. Protein translocation and thylakoid biogenesis in cyanobacteria. Biochimica et Biophysica Acta (BBA) - Bioenergetics [Internet]. 2016 Mar [cited 2020 Apr];1857(3):266-73. Available from: https://www.sciencedirect.com/science/article/pii/S0005272815001747

Kamennaya NA, Kennaway G, Fuchs BM and Zubkov MV. Pomacytosis-semi-extracellular phagocytosis of cyanobacteria by the smallest marine algae. PLOS Biology [Internet]. 2018 Jan 5 [cited 2020 Apr]. Available from: https://journals.plos.org/plosbiology/article?id=10.1371%2Fjournal.pbio.2003502

Kim J-S, Lee H-J, Kim S-K and Kim H-J. Global pattern of Microplastics (MPs) in commercial food-grade salts: sea salt as an indicator of seawater MP pollution. Environ. Scie Tech. [Internet]. 2018 Oct 4 [cited 2020 Apr];52(21):12819-28. Available from: https://pubs.acs.org/doi/10.1021/acs.est.8b04180#,

Kusuma DW, Murdimanto A, Aden LY, Sukresno B, Jatisworo D andHanintyo.. Sea surface temperature dynamics in Indonesia. IOP Conference Series: Earth and Environmental Science [Internet]. 2017 [cited 2020 Apr];98. Available from: https://iopscience.iop.org/article/10.1088/1755-1315/98/1/012038

Lachmann, Florina & Carney Almroth, Bethanie & Baumann, Henrikke & Broström, Göran & Corvellec, Hervé & Gipperth, Lena & Hassellöv, Martin & Karlsson, Therese & Nilsson, Per. (2017). Marine plastic litter on Small Island Developing States (SIDS): Impacts and measures. 10.13140/RG.2.2.16168.37121

Lambert T. Superfolder GFP at FPbase [Internet]. FPbase; c2020 [cited 2020 Apr]. Available from: https://www.fpbase.org/protein/superfolder-gfp/

Martin, L. (2019, July 24). What Are the Roles of Chlorophyll A & B? Retrieved October 18, 2020, from https://sciencing.com/what-are-the-roles-of-chlorophyll-a-b-12526386.html

Mason SA, Welch VG and Neratko J. Synthetic polymer contamination in bottled water. Front Chem [Internet]. 2018 Sep 11 [cited 2020 Apr];6:407. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6141690/

McGregor D. 10 interesting facts about the Great Pacific Garbage Patch [Internet]. Eradicate Plastic; [cited 2020 Apr]. Available from: http://eradicateplastic.com/10-interesting-facts-about-the-great-pacific-garbage-patch/

Nurkolis, Nuryadin D, Syaifudin N, Handika R, Hendroko R and Udjianto DWI. The economic of marine sector in Indonesia. Aquatic Procedia [Internet]. 2016 Aug [cited 2020 Apr];7:181-6. Available from: https://www.researchgate.net/publication/306096590_The_Economic_of_Marine_Sector_in_Indonesia

Parker L. The world's plastic pollution crisis explained [Internet]. Washington (DC): National Geographic Partners LLC; c1995-2020 [accessed 2020 Apr 24]. Available from: https://www.nationalgeographic.com/environment/habitats/plastic-pollution/

Periplasm [Internet]. Encyclopedia.com; 2020 [cited 2020 Apr]. Available from: https://www.encyclopedia.com/science-and-technology/biology-and-genetics/cell-biolog/periplasm

Photosynthesis in bacteria [Internet]. Photosynthesis Education; [cited 2020 Apr]. Available from: https://photosynthesiseducation.com/photosynthesis-in-bacteria/

Putri MR, Setiawan A and Safitri M. Variation of ocean pH in the Indonesia waters. AIP Conference Proceedings [Internet]. 2015 Sep 30 [cited 2020 Apr];1677(1):060021. doi: 10.1063/1.4930701

Singh SB, Young K and Silver LL.. What is an "ideal" antibiotic? Discovery Challenges and path forward. Biochem Pharmacol [Internet]. 2017 Jun 1 [cited 2020 Apr];133:63-74. Available from: https://www.ncbi.nlm.nih.gov/pubmed/28087253

Soo RM, Hemp J, Parks DH, Fischer WW and Hugneholtz P. On the origins of oxygenic photosynthesis and aerobic respiration in cyanobacteria. Science [Internet]. 2017 Mar 31 [cited 2020 Apr];355(6332):1436-40. Available from: https://pubmed.ncbi.nlm.nih.gov/28360330/

UniProt. ISF6_0224 - Mono(2-Hydroxyethyl) Terephthalate Hydrolase Precursor - Ideonella Sakaiensis (Strain NBRC 110686 / TISTR 2288 / 201-F6) - ISF6_0224 Gene & Protein [Internet]. UniProt; 2020a [cited 2020 Apr]. Available from: https://www.uniprot.org/uniprot/A0A0K8P8E7

UniProt. ISF6_4831 - Poly(Ethylene Terephthalate) Hydrolase Precursor - Ideonella Sakaiensis (Strain NBRC 110686 / TISTR 2288 / 201-F6) - ISF6_4831 Gene & Protein [Internet]. UniProt; 2020b [cited 2020 Apr]. Available from: https://www.uniprot.org/uniprot/A0A0K8P6T7

What are the roles of chlorophyll A & B?. Sciencing, 2020, https://sciencing.com/what-are-the-roles-of-chlorophyll-a-b-12526386.html

Wilson A and Killinger J. Plastics 101 [Internet]. Washington (DC): American Chemistry Council, Inc.; c2005-2020 [cited 2020 Apr]. Available from: https://plastics.americanchemistry.com/Plastics-101/