Understanding the mechanisms of small-scale composting and potential enzymatic improvements using synthetic biology

Shada Aborawi, Rebecca Avileli, Jasmine Belisle, Thomas Byrne, Wenyu Chen, Kimoya Edwards, Olive Graham, Linda He, Livia Kadezabek, Charlin Li, Dominic Piper, Declan Sander, Lana VanGenderen, Elisha Wong, Marissa Wong, Michelle Wu

Catholic Central High School, Lethbridge Collegiate Institute, Chinook High School, Winston Churchill High School

Reviewed on 2 May 2020; Accepted on 22 June 2020; Published on 26 October 2020

As the population of the earth continues to grow, more waste is ending up in landfills, with a large portion of the items occupying the landfill space being compostable food waste. When food waste enters landfills, there are a variety of environmental consequences such as the copious amounts of greenhouse gases emitted during the production of our food as well as the anaerobic decompositions that occur in landfills. Additionally, food shipping often utilizes cardboard and plastic, which poses harm to the environment. Although food waste disposal services are available, such services release methane and are not economically feasible. Furthermore, both residential and industrial composting facilities do assist in combating the issue of food waste but are oftentimes not readily available. Therefore, we propose a system that employs an engineered biological catalyst that breaks down food waste in a small-scale closed container system, suitable for keeping animals out, decreasing the need for space in landfills and ensuring that unsafe chemicals produced during the composting do not enter the environment. Our aim is to use readily available household materials to design an efficient small-scale composting unit, while increasing the speed of decomposition using an engineered pectin-degradation enzyme catalyst. This project will not only address a local problem within the Lethbridge community, where no city-wide composting system exists, but can also be expanded to other communities where these services are not available.

Keywords: Composting, pectin, homogalacturonan

Mentors: Laura Keffer-Wilkes, Jalyce Heller, Damian La Rosa Montes, Dia Michailidou-Koupantsis, Luke Saville

Direct correspondence to kefferwilkesl@uleth.ca; jalyce.heller@uleth.ca

Watch a video introduction by the authors at https://tool.animaker.com/animo/QVHtUatLWpnJ

Background

Food waste, a significant yet commonly misunderstood problem is a major contributor to climate change. Currently, methane and other greenhouse gases that are released by food that is undergoing anaerobic decomposition in landfills make up about 8% of global greenhouse gas emissions (Frischmann 2018). A startling 1.6 billion tonnes of food waste is produced on a global scale annually, as predicted by the Food and Agricultural Organization of the United Nations (Food and Agriculture Organization 2020) and current methods to improve this are not very widespread and efficient.

The biochemical process of composting

One of the major ways in which food waste is mitigated is through the process of composting. In its most basic form, compost is produced when organic and nitrogenous waste is digested by decomposers (e.g. insects, fungi, and bacteria), resulting in the production of thermal energy, carbon dioxide and water (Midwest Laboratories 2016). Under ideal conditions of a 30:1 carbon:nitrogen ratio, a neutral pH between 5.5 and 8.5, bulk density of less than 415 kg per cubic meter, and at least 5% available oxygen concentration, the result will be nutrient dense rich black earth, that has decreased dramatically in mass and size (Power, Dick, Kashmanian, et al. 2000).

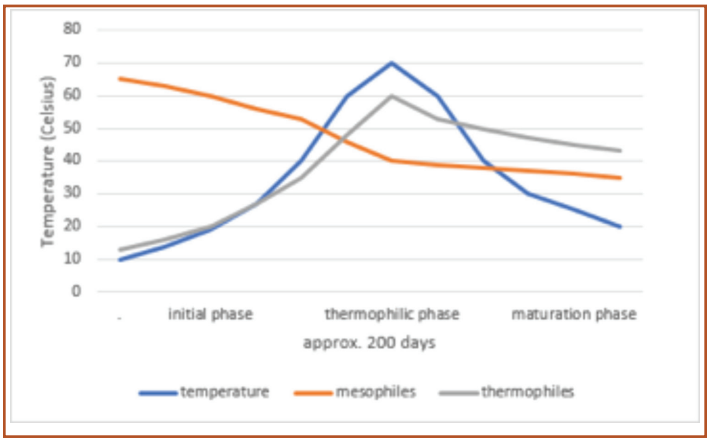


Figure 1. *The stages of composting. Over the course of about 200 days, compost cycles through three stages that can be defined by the temperature and the bacterial composition of the*

Compost goes through three main stages over time. The initial stage consists mainly of physical decomposition by insects and large organisms, causing rapid decomposition of amino acids. In addition, as seen in Figure 1, some thermophilic bacteria such as Actinomycetes begin to actively break down the more complex compounds (e.g. cellulose, lignin, chitin, and proteins). As bacterial activity increases, more organic matter is broken down thus raising the temperature of the compost (CalRecycle, 2018).

As the compost reaches the thermophilic phase, the majority of decomposition occurs, as it is the longest stage with the most active bacteria. This is the point where the cellulose, hemicellulose, and other parts of the plant cell walls are broken down (Joseph 2019). Once the compost reaches a high enough temperature, the harmful pathogens are killed, sanitizing it. At this point, the compost is turned over either by turning the soil or the container to avoid it reaching temperatures above 70 °C, in which the thermophilic bacteria would die, slowing or even stopping the process all together (CalRecycle 2018).

The third and final maturation phase occurs after the compost has been turned. There is no longer enough food for the bacteria, causing them to die off. This is when there will be the most fungal activity in the compost, which will finish the process of decomposing cellulose and lignin. The result is a homogeneous soil conditioner around half the weight of the original waste, composed of mineral and bacterial matter, and high in organic content (Midwest Laboratories 2016, Khater 2015).

Problems with current methods

Many individuals are turning to small-scale home composting to help combat environmental issues, but this is only efficient in certain climates due to its specific optimal environmental needs. Because of Southern Alberta's dry, windy and cold weather, home composting is not an option for many as it would take a longer time due to lack of optimal conditions required for the organic and biochemical process of composting. The weather proposes a high risk for home composting as not everyone is knowledgeable about how to combat these suboptimal conditions. The weather is unpredictable and not everyone has the resources to control the small possibilities. We aim to bring in a local factor to our solution as it could make composting in Southern Alberta a faster and more efficient process despite the dry and cold weather. Due to the dry and cold weather, home composting is more challenging and takes more time to complete where if not done properly, it can attract a variety of pests which can cause many complications (Olney 2015). These persistent issues make it easier for the general public to dispose of their food waste by throwing it in the garbage unless they are in cities that have access to composting facilities. Within these composting facilities, pre-consumer food waste and post-consumer food waste are often separated and shredded to speed up the decomposition process (How the Calgary composting facility works 2020) upon their arrival. For

the first 21 days (How the Calgary composting facility works 2020), the materials are transferred to composting vessels where temperature, moisture content and oxygen levels are all monitored to ensure an optimal environment. After these 21 days, the compost is screened for any contaminants and then moved to a curing building for another 21 days. During this time, the compost is turned every five days to introduce oxygen and encourage decomposition. Once the days are up, the compost is again tested for safety before distribution.

The pectin problem

Due to shortcomings in the current methods of composting, such as inaccessibility to composting facilities in smaller jurisdictions, and the challenges associated with composting facilities themselves, our project is based on increasing the efficiency and accessibility of small-scale home composting. We hope to accomplish this through engineering a microbe to break down pectin in compost, thus accelerating the process. Pectin is a structurally complex family of polysaccharides (Mohnen 2008), found in the cell walls and intercellular tissues (Tikkanen 2007) of vascular plants, as well as in the walls of gymnosperms, bryophytes, pteridophytes, and Chara (Mohnen 2008). Pectin helps crosslink hemicellulose and cellulose fibres, to improve the rigidity of the cell walls (Caspi 2013). Pectin is soluble, therefore extractable, in both aqueous or dilute acidic conditions (Braidwood, Breuer and Sugimoto 2014, Tikkanen 2007). Since pectin is able to produce a viscous gel-like solution, it is often used to make jams, jellies, and marmalades; this thickening property is also utilized for pharmaceutical and confectionary purposes, as well as in the textile industry (Tikkanen 2007).

Because pectin is present in the majority of the upper plants and fruits that make up a compost pile, targeting the breakdown of pectin will increase the rate of composting. There are a variety of pectins, which vary in structural complexity, but the one that is the most abundant in nature is homogalacturonan (HG). HG forms the backbone to most pectins, making it the simplest pectic polysaccharide (Mohnen D 2008., Caspi 2013). We will be primarily focusing on breaking down HG for our system due to its commonality and structural simplicity.

Systems level

For our system we are focussing on the degradation of HG. We will use the process of pectin degradation as a basis and outline for food waste to be broken down more efficiently. We will engineer Escherichia coli to host and produce unsaturated glucuronidase (YteR), polygalacturonase (Peh), pectin methylesterase (Pem) and pectin lyase (Pnl) which will speed up the process and enable everyone to compost safely.

Our system will follow the degradation pathway of HG by mimicking the process done by indigenous plant enzymes. Degradation of HG by plant enzymes involves both pectinase and endo-Peh which attacks the pectin chain at random positions from the middle of the enzyme. Methylesterase-specific pectinase enzymes remove the methyl groups from the HG in the form of methanol and prepare the de-esterified domains of the HG for further processing. These de-esterified chains are now suitable for the use of the endo-Peh which if crosslinked with calcium will result in gel formation (Caspi 2018).The pectinase will remove the methyl groups making a de-esterified molecule called pectate (Caspi 2018).

To increase efficiency in composting we propose a system composed of an easy-to-use at-home composting bin along with lyophilized engineered enzymes made of an HG degradation pathway engineered in a host candidate. Members of our team will follow a DIY home composting protocol to build our own composter. The materials that we will use are simple items that can be found in a typical household such as a plastic container with a lid, nylons, glue, soil, newspaper, and a wooden spoon. The methods involve making five holes around two centimeters in diameter in the lid to allow for aeration, followed by using a piece of nylon to create a screen glued to the underside of the lid; preventing bugs from entering. Lastly, soil and shredded newspaper will be added. Each member will then turn their compost weekly with a wooden spoon to aerate it. Additionally, we will each record the food waste we are putting in the compost, as well as observe

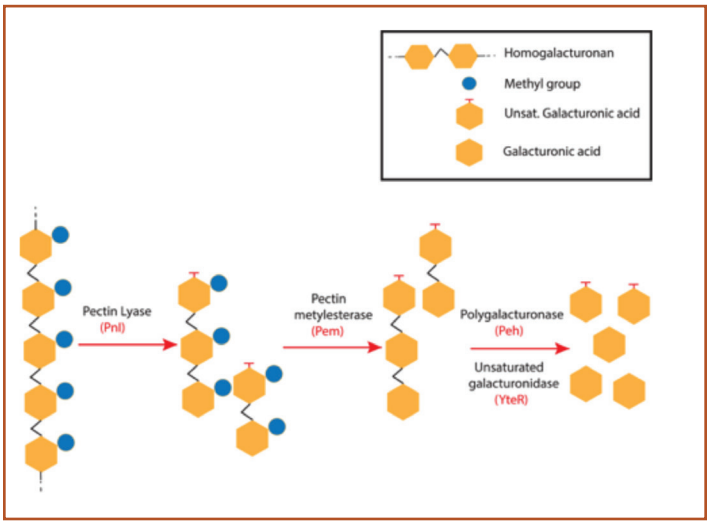


Figure 2. *Proposed homogalacturonan degradation using Pnl, Pem, Peh and YteR.*

and record the temperature, moisture, weight of waste, pH, nutrient decomposition, smell, and salt levels on observation tables. The devices we will use during this process involve a thermometer (temperature), weight of waste (scale), smell (based on if the area around the composter smells putrid or like rotten eggs), and nutrient decomposition (a before and after weight measure of the soil). For further analysis we will set aside a small sample of the compost weekly and store it at -20 oC for future testing.

Although HG is the simplest pectin, its degradation requires multiple enzymes. As said above, our system will focus on the indigenous pathway of HG degradation with Pnl, Pem, YteR and PeH until galacturonic acid (GalA) monomers are formed (Figure 2).

With a focus on HG, our system will use a combination of enzymes to accelerate its breakdown from a linear polymer into monomers. We are considering two different approaches in the delivery of our system. The first being to engineer an HG degradation pathway into a microbe, then we will apply the lysate to compost either in spray form or as a built in factor to the composter. Second, we could purify each of our enzymes and create a lyophilized powder. Our system's ability to ensure a rapid degradation of the pectin HG suggests a promising advancement to present day composting. Our system provides a convenient addition to any beginner compost, it resolves time constraints, and is more efficient.

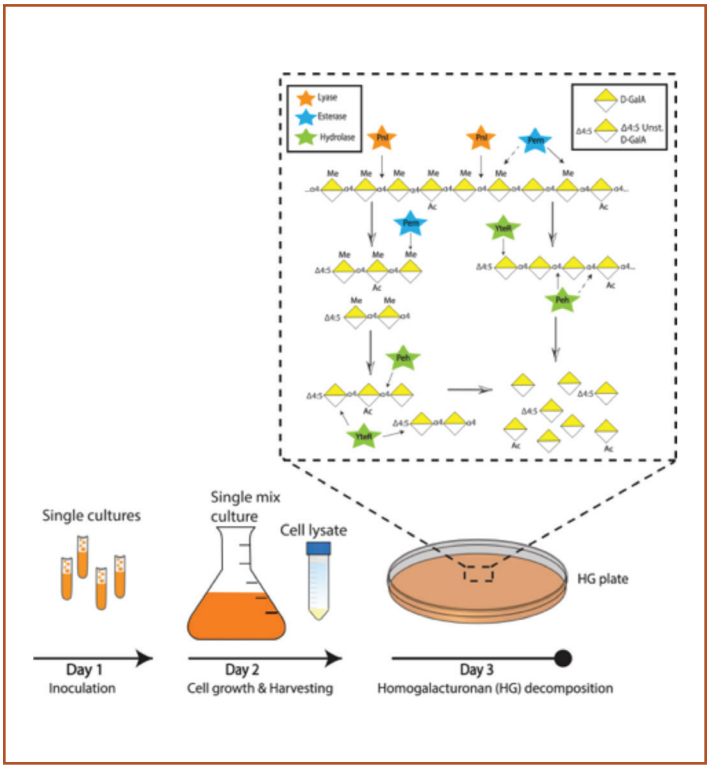


Figure 3. *Reconstitution of homogalacturonan (HG) deconstruction cell-free system. Day 1 and 2, cell culture, protein expression and reconstitution of the cell-free system. Day 3, validation of the in-house HG degradation assay. Hypothesized deconstruction mechanism by selected pectinases. Pnl, pectin lyase; Pem, pectin methylesterase; YteR, unsaturated glucuronidase; Peh, polygalacturonase.*

Device level

Homogalacturonan deconstruction cell-free system

Our approach is to generate an HG decomposition cell-free system reconstituted from cell lysate or purified components strategically selected from Paenibacillus amylolyticus (P. amylolyticus) genome (Figure 3). These are advantageous to our system because both help to minimize degradation of cells and increase the amount of reproducibility possible for cells. The cell-free system will be composed of Pnl, Pem, YteR and Peh. The Pnl will cleave extensively methylated GalA with high efficiency within a wide range of temperature (Temp opt = 55 °C). On the other hand, Pem will demethylate GalA, allowing YteR and Peh to further cleave any unsaturated and demethylated HG into smaller fragments. The amount, pH and temperature are some of the conditions that will be monitored to ensure that the selected pectinases are at their best optimal functioning. This serves to enhance the thermostability of the whole cascade essentially contributing to a more flexible and efficient home composting system.

Organism selection

When selecting the host organism to express the recombinant pectinases for our HG degradation assay, the chemical properties as well as biosafety and ethical concerns associated with the host must be considered. This is because the optimal goal of our system is to be used daily in small-scale home composting. Thus, numerous measurements must be taken to reduce the presence of any undesired host molecule that could endanger human health, jeopardizing the safety and ethical approval of our system for common household usage.

A great host for our system is E. coli. It is presently the best understood and most widely used organism in molecular and synthetic biology. It exhibits rapid growth and high protein yields, which along with the extensive genetic toolbox available, provides a model organism to study bacterial metabolic pathways. More importantly, its genome does not have any pectin-degrading enzymes,

making it a great candidate to characterize and validate our chosen HG degradation mechanism. Thus, E. coli strains with high transformation efficiency such as DH5α will be used for plasmid amplification while the strain BL21(DE3) will be utilized to express the engineered recombinant pectinases of our system.

On the other hand, *Bacillus subtilis* can be used as a possible host in the future. Its rapid growth on chemically defined media, straightforward genetic manipulation coupled with its ability to produce and secrete high levels of proteins have made *B. subtilis* an important bacterial platform for numerous industrial applications. More importantly, it is classified as safe, and not considered pathogenic or endotoxic to humans (Elshaghabee, Rokana, Gulhane, et al. 2017). Thus, it is commonly used as a probiotic supplement to boost humans' metabolic system (Lefevre, Racedo, Denayrolles, et al. 2017). However, certain strains are able to utilize many plant cell wall polysaccharides such as pectin as the carbon source (Ochiai, Itoh, Kawamata, et al. 2007), indicating the presence of a significant list of pectinases in its genome (Keggi and Doran-Peterson 2019). Although, this could potentially increase the effectiveness of pectin degradation, it would make the characterization and validation of our in-house HG decomposition cell-free system quite challenging (Figure 3).

Gene expression in bacteria

Once plasmids carrying the optimal protein sequences have been transformed into the E. coli, protein expression will be induced. This occurs through the addition of IPTG (Isopropyl β-D-1-thiogalactopyranoside), a reagent that interacts with the promoter sequence to stimulate protein expression. Ideally this will result in gene overexpression, which will lead to the high expression of the target proteins compared to the rest of the bacterial proteins. Even though IPTG would be great for expression of the protein gene sequences, it is very expensive and not easily acquirable.

Pectin degradation

To test the effectiveness of our system, the cell lysate or purified proteins will be applied to HG with different degrees of methylation. Both the amount of cell lysate or purified proteins and HG pectin will be quantified and controlled, to accurately carry out experiments. A colourimetric assay will be conducted to assess the degradation abilities of our system. An increase in absorbance should correlate with an increase in galacturonate monomer formation. Meaning that the more HG that is broken down, the higher the absorbance should be at the specified wavelength.

Parts level

Pectin

Our project is focused on engineering enzymes that will degrade the pectin HG. So far, we believe that ours will be the first iGEM project based on HG degradation. HG is found in nearly all fruits and vegetables and helps keep plant tissue firm and are found in the structure of the plant cells.

Pectin lyase pamy_2278 (Pnl)

This is an enzyme that degrades pectin and cleaves methylated monomers (Bonnin E, 2014). It is commonly found in fungi such as Aspergillus flavus (Bonnin, Ralet, Thibault, et al. 2014). Although Pnl are commonly found in microorganisms, they can also be found in plants and animals (Bonnin, Ralet, Thibault, et al. 2014).

Pectin methylesterase pamy_4273 (Pem)

Pem is responsible for the removal of methyl esters in the cell wall of plant cells (Wu, Bulgakov and Jinn 2018). It is found in *B. subtilis* (Sadana 1998, Microchem Laboratory 2014).

Polygalacturonase pamy_82 (PeH)

This enzyme catalyzes the hydrolysis of α-1,4 glycosidic bonds between PGA, yielding d-galacturonate, a monomer (Singh, Kundu, Das, et al. 2019). It is found in bacteria, fungi, plants and animals (Sussman, Amin, Yoder, et al. 2018).

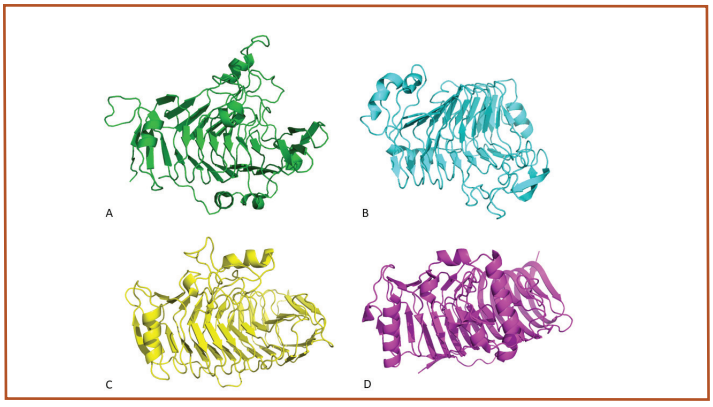


Figure 4. *Structural representatives of PemA (A; PDB 1QJV), PnL (B; PDB 1IDJ), PeH (C; 1BHE) and YteR (D; PDB 5OLP), show the similar β-sheet formation.*

Unsaturated glucuronidase pamy_1066 (YteR)

This enzyme catalyzes hydrolysis in carbon bonds creating monomers (Stevenson 2019). YteR has been

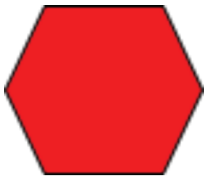
found in bacteria such as *B. subtilis* and *Bacteroides thetaiotaomicron* (Sussman, Amin, Yoder, et al. 2018).

All four proteins within this pathway share a similar protein structure (Figure 4), comprising a β-sheet, common among pectate lyase, pectin lyase, polygalacturonase and rhamnogalacturonan enzymes (Jenkins, Mayans, Smith, et al. 2001).

Constructs

DNA constructs will be composed of the enzymatic genes in plasmids with the following regulators: an IPTG inducible promoter, a ribosomal binding site and double terminator (Table 1). Constructs will begin with the T7 promoter (BBa_I712074) from the iGEM registry, followed by the ribosomal binding site (BBa_J61100), the gene for the selected enzyme and finally the double terminator (BBa_B0014) (Figures 5).

Table 1. *Symbols denoting different parts of each gene circuit.*

T7 PROMOTER	RIBOSOMAL BINDING SITE	DOUBLE TERMINATOR
BBa_I712074	BBa_J61100	BBa_B0014
		

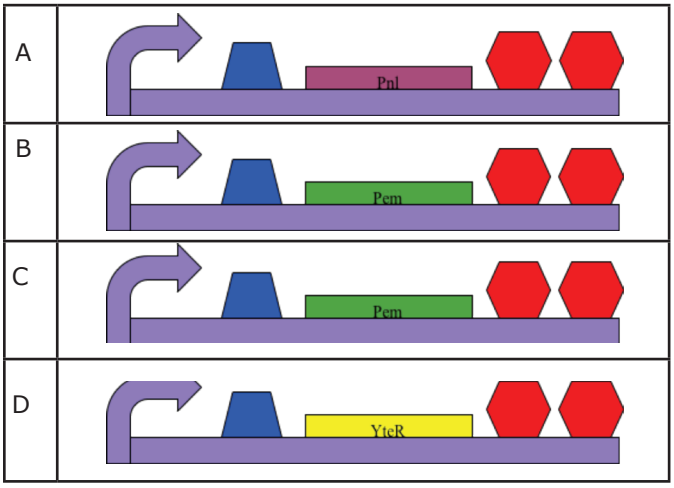


Figure 5. *Genetic constructs for (A) pectin lyase, (B) pectin methylesterase, (C) polygalacturonase, and (D) unsaturated galacturonidase.*

Safety

When genetically engineering enzymes to degrade pectin, safety is critical. If the wrong gene is used or placed in the wrong location, a defective enzyme can be created and be harmful for those in the lab. Safety precautions must be ensured because even in a lab and especially one with genetic engineering where even one uncontrolled factor can cause untold disaster and harm. We have ensured that we have the proper training to take safety precautions in the lab. Everyone has completed their Workplace Hazardous Materials Information System (WHMIS) Certification. Problems can arise if compost is not managed correctly, such as smell, leachate, flies, and rodents (Tuladhar and Spuhler 2020). If the compost does not have enough oxygen and aeration, anaerobic respiration can occur. This results in sulfur and other odorous compounds to form, creating odor pollution. These odors produced in the composting process can lead to high symptom prevalence, like affective, gastrointestinal, head-related, cardiac, cognitive, neuromuscular, and musculoskeletal symptoms (Zhu, Zheng, Gao, et al. 2016). Another potential concern with small-scale home composting is the possible existence of human pathogens. If self-heating occurs and is managed properly in a large compost pathogen reduction can take place. In a small compost achieving a high temperature is often not accomplished, thus increasing the potential for the survival of pathogens (Harrison, Olmstead, Bonhotal, et al. 2004). However, when practicing proper care methods the relative health risks in small-scale at-home composting is low, but pets and small children should be kept away to avoid swallowing and unnecessary provocation of compost. Gloves can be worn to control how much bacteria is exposed to the skin.

When creating a new system by using engineered microbes, we have kept in mind that there may be issues and errors causing adverse effects. There is a possibility that we could mistake an unsuspected target as the correct one, leading to mutations which may cause the system to lose the appropriate function (Prakash, Bhatia, Verma, et al. 2011). By engineering pectin degrading enzymes in a lab we have the ability to grow more than what would naturally occur in a compost pile, and by doing this we also gain the advantage of eliminating some worry about bacterial outbreak. Examples of such enzymes being Pnl, Pem, Peh,YteR.

Discussions

Increasing compost rates by creating a microbe with a pectin degradation pathway for HG does not increase degradation of other compostable components.The effectiveness of our proposed microbe is limited, to an extent, as it will be unable to degrade complex pectins, or other components of compost such as proteins and fats. To degrade HG, methylesterase is required through a process by removing methyl groups in order to cleave HG into smaller pieces with other extracellular enzymes. This allows HG to enter into the cell for complete degradation (Frischmann 2018). Microbial produced pectinases require conditions with alkaline environments and high temperatures, so maintaining these environments will improve the efficiency of our system. For future directions, our team will be looking into improving the thermostability of our enzymes by using bioinformatics tools to identify amino acids that can be mutated, and then performing molecular dynamics simulations to assess the folding and stability of these mutants at high temperatures.

Ultimately, the goal of our project is to make home composting a viable alternative to throwing away food waste in garbage disposals. This means that our system must be cost-effective, as we want it to be affordable for everyone. Our team will need to communicate with members of our community to gather their opinions on how much money an average person or business would be willing to spend on a composting kit. Additionally, our system needs to address current roadblocks preventing people from home composting, namely, that home composting is simply too inefficient to degrade the food waste created by the modern household. The team has set up home composting stations in which we can measure variables such as temperature, moisture, weight of waste, pH, nutrient decomposition, and salt levels. We will use these composters to gather data about rates of compost unaided by our system, and compare these with the rates of composting after the addition of our system (once we have successfully created and tested this system in the lab). If our system causes the rates of composting to increase, that would indicate that our project is successful. This will also show us the conditions under which our system is the most effective. Data collection for variables such as different households or environments has not been discussed as of yet but will be in the future.

Food waste is a major global problem that squanders the resources that go into food production, and results in gas emissions that contribute to global warming. Composting helps to solve this issue in two ways: it diverts food waste away from the landfill, and it provides nutrient-rich soil for community members to use for growing their own food. We hope to raise awareness about the importance of composting, and to utilize synthetic biology to create a compost accelerating system that can be used by our community, and eventually others, to aid in their composting efforts.

Acknowledgements

The Lethbridge High School iGEM team acknowledges the support of the geekstarter & Mindfuel Programs, the University of Lethbridge Department of Chemistry & Biochemistry, and team advisors Dr. Laura Keffer-Wilkes, Jalyce Heller, Luke Saville, Dia Michailidou-Koupantsis, and Damian La Rosa Montes.

References

Anderson J. Part:BBa J61100 [Internet]. Boston (MA): International Genetically Engineered Machine, Registry of Standard Biological Parts; 2007 Jan 28 [cited 2020 Apr]. Available from: https://parts.igem.org/Part:BBa_J61100

Bonnin E, Ralet M-C, Thibault J-F and Schols HA. Enzymes for the valorisation of fruit- and vegetable-based co-products [Internet]. Handbook of Waste Management and Co-Product Recovery in Food Processing. Woodhead Publishing; 2014 Mar 27 [cited 2020 Apr 22]; pp. 257-85. Available from: https://www.sciencedirect.com/science/article/pii/B9781845693916500127

Braidwood L, Breuer C and Sugimoto K. My body is a cage: mechanisms and modulation of plant cell growth. New Phytologist Trust [Internet]. 2014 Jan [cited 2020 Apr 19];201(2):388-402. Available from: https://nph.onlinelibrary.wiley.com/doi/full/10.1111/nph.12473

CalRecycle. Compost pile microbes [Internet]. (CA): California Department of Resources Recycling and Recovery, Organic Materials; 2018 Dec 7 [cited 2020 Apr 19]. Available from: https://www.calrecycle.ca.gov/organics/homecompost/microbes

Caspi R. MetaCyc pathway: homogalacturonan degradation [Internet]. Menlo Park (CA): SRI International; 2018 Jun 7 [cited 2020 Apr]. Available from: https://biocyc.org/META/NEW-IMAGE?type=PATHWAY&object=PWY-1081

Caspi R. MetaCyc Pathway: pectin degradation II [Internet]. Menlo Park (CA): SRI International; 2013

Mar 14 [cited 2020 Apr 19]. Available from: https://biocyc.org/META/NEW-IMAGE?type=PATHWAY&object=PWY-7248

Elshaghabee FMF, Rokana N, Gulhane RD, Sharma C, and Panwar H. Bacillus as potential probiotics: status, concerns, and future perspectives. Front Microbiol [Internet]. 2017 Aug 10 [cited 2020 Apr];8:1490. Available from: https://pubmed.ncbi.nlm.nih.gov/28848511/

Food and Agriculture Organization. Food wastage: key facts and figures [Internet]. Italy: Food and Agriculture Organization of the United Nations; c2020 [cited 2020 Apr 19]. Available from: http://www.fao.org/news/story/en/item/196402/icode/

Frischmann C. Opinion: The climate impact of the food in the back of your fridge. The Washington Post [Internet]. 2018 Jul 31 [cited 2020 Apr 19]. Available from: https://www.washingtonpost.com/news/theworldpost/wp/2018/07/31/food-waste/

Gaber R. Part:BBa I712074 [Internet]. Boston (MA): International Genetically Engineered Machine, Registry of Standard Biological Parts; 2007 Oct 2 [cited 2020 Apr]. Available from: https://parts.igem.org/Part:BBa_I712074

Harrison E, Olmstead D, Bonhotal J and Regenstein JM. Health and safety guidance for small scale composting. Cornell Waste Management Institute [Internet]. Department of Crop and Soil Sciences Cornell University; 2004 [cited 2020 April 22]. Available from: p://cwmi.css.cornell.edu/smallscaleguidance.pdf

Jenkins J, Mayans O, Smith D, Worboys K and Pickersgill RW. Three-dimensional structure of Erwinia chrysanthemi pectin methylesterase reveals a novel esterase active site. J Mol Biol [Internet]. 2001 Jan 26 [cited 2020 Apr 27];305(4):951-60. Available from: https://www.ncbi.nlm.nih.gov/pubmed/11162105

Joseph K. The science of compost [Internet]. Untamed Science; 2019 Jan [cited 2020 Apr 18]. Available from: https://untamedscience.com/biology/ecology/ecology-articles/the-science-of-compost/

Keggi C and Doran-Peterson J. Paenibacillus amylolyticus 27C64 has a diverse set of carbohydrate-active enzymes and complete pectin deconstruction system. Journal of industrial microbiology & biotechnology. 2019;46(1):1-11.

Khater ESG. Some physical and chemical properties of compost. International Journal of Waste Resources [Internet]. 2015 Feb 24 [cited 2020Apr19];5(1):1–5. Available from: https://www.longdom.org/open-access/some-physical-and-chemical-properties-of-compost-2252-5211-1000172.pdf

Lefevre M, Racedo SM, Denayrolles M, Ripert G, Desfougères T, Lobach AR, Simon R, Pélerin F, Jüsten P and Urdaci MC. Safety assessment of *Bacillus subtilis* CU1 for use as a probiotic in humans. Regulatory Toxicology and Pharmacology. 2017 Feb;83: 54-65.

Microchem Laboratory. *Bacillus subtilis* [Internet]. Round Rock (TX): Microchem Laboratory; 2014 [cited 2020 Apr 25]. Available from: https://microchemlab.com/microorganisms/bacillus-subtilis

Midwest Laboratories. The process and chemistry of composting [Internet]. Omaha (NE): Midwest Laboratories; 2016 [cited 2020 Apr 19]. Available from: https://midwestlabs.com/wp-content/uploads/2016/11/156-Process-and-Chemistry-of-Composting.pdf

Mohnen D. Pectin structure and biosynthesis. Current Opinion in Plant Biology. 2008;11(3):266–77.

Ochiai A, Itoh T, Kawamata A, Hashimoto W and Murata K. Plant cell wall degradation by saprophytic *Bacillus subtilis* strains: gene clusters responsible for rhamnogalacturonan depolymerization. Appl. Environ. Microbiol. 2007;73(12):3803-13.

Olney K. How to compost without attracting pests [Internet]. SafeBee; 2015 Jun 8 [cited 2020 Apr 19]. Available from: http://www.safebee.com/home/how-compost-without-attracting-pests

Power JF, Dick WA, Kashmanian RM, Sims JT, Wright RJ, Dawson MD and Bezdicek D, editors. Land application of agricultural, industrial, and municipal by-products [Internet]. Vol. 6. Madison, WI: Soil Science Society of America; 2000 Jan 1 [cited 2020 Apr 19]. Available from: https://acsess.onlinelibrary.wiley.com/doi/book/10.2136/sssabookser6

Prakash D, Bhatia R, Verma S and Tinwary BN. Risks and precautions of genetically modified organisms. International Scholarly Research Notices [Internet]. 2011 Nov 22 [cited 2020 April 23]. Available from: https://www.hindawi.com/journals/isrn/2011/369573/

Sadana A. High-resolution fractionation processes. Separation Science and Technology [Internet]. Academic Press; 1998 [cited 2020 Apr 22];1:61-99. Available from:

https://www.sciencedirect.com/science/article/pii/S0149639598800317

Shetty R. Part:BBa B0014 [Internet]. Boston (MA): International Genetically Engineered Machine, Registry of Standard Biological Parts; 2003 Jul 16 [cited 2020 Apr]. Available from: https://parts.igem.org/Part:BBa_B0014

Singh J, Kundu D, Das M and Banerjee R. Enzymes in food biotechnology: production, application and future prospects [Internet]. Academic Press; c2019. Chapter 24, Enzymatic processing of juice from fruits/vegetables: an emerging trend and cutting edge research in food biotechnology; [cited 2020 Apr 25]. Available from: https://www.sciencedirect.com/science/article/pii/B9780128132807000244

Stevenson J. Glycoside hydrolase family 105 [Internet]. CAZypedia; 2019 Oct 27 [cited 2020 Apr 22]. Available from: https://www.cazypedia.org/index.php/Glycoside_Hydrolase_Family_105

Sussman J, Amin K, Yoder M, Harel M and Prilusky J. Polygalacturonase [Internet]. Israel: Weizmann Institue of Science, Proteopedia: life in 3D; 2018 Aug 27 [cited 2020 Apr 22]. Available from: http://proteopedia.org/wiki/index.php/Polygalacturonase

Tikkanen A, Pectin [Internet]. Britannica Academic. Encyclopædia Britannica; 2007 [cited 2020Apr19]. Available from: https://academic-eb-com.ezproxy.uleth.ca/levels/collegiate/article/pectin/58914

Tuladhar B and Spuhler D.(Co-) composting (Small-scale): Factsheet [Internet]. Sustainable Sanitation and Waste Management Toolbox. 2020 [cited 2020 Apr 22]. Available from: https://sswm.info/sswm-solutions-bop-markets/affordable-wash-services-and-products/affordable-technologies-and/%28co-%29-composting-%28small-scale%29

How the Calgary composting facility works [Internet]. The City of Calgary; c2020 [cited 2020 Apr 19]. Available from: https://www.calgary.ca/UEP/WRS/Pages/Recycling-information/Residential-services/Green-cart/Green-Cart-organics-composting-facility.aspx

Wu H-C, Bulgakov VP, Jin T-L. Pectin Methylesterases: cell wall remodeling proteins are required for plant response to heat stress. Front Plant Sci [Internet]. 2018 [cited 2020 Apr 25]. Available from: https://www.frontiersin.org/articles/10.3389/fpls.2018.01612/full

Zhu Y-L, Zheng G-D, Gao D, Chen T-B,Wu F-K, Niu M-J and Zou K-H. Odor composition analysis and odor indicator selection during sewage sludge composting. J Air Waste Manag Assoc [Internet]. 2016 May 18 [cited 2020 Apr 22]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5062037/