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United States Department of Agriculture
Project Initiation

Project Data for Accession Number 1034628

Project Title

Investigating secondary stressors in PHA production and increasing variety of PHA produced from dairy and paper waste

Project Details

Sponsoring Institution National Institute of Food and Agriculture	**Project Status** ACTIVE
Funding Source NIFA Non Formula	**Grant Year** 2026
Grant No. 2026-67011-46314	**Cumulative Award Amt.** $180,000.00
Proposal No. 2024-13170	**Multistate No.** (N/A)
Project Start Date May 01, 2026	**Project End Date** Apr 30, 2029
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(N/A)

Non Technical Summary

Project Summary: Polyhydroxyalkanoates (PHAs), a type of polyester synthesized by various bacteria and archaea under nutrient-limited or other stressful conditions, offer a promising biodegradable alternative to conventional plastics. However, their production costs remain too high to compete with petrochemical-based plastics. Increasing both the types of PHAs produced and strategies for increasing PHA yield will help lower this cost. Producing more versatile PHA co-polymers, such as polyhydroxybutyrate-co-polylactic acid (PHB-co-PLA), will contribute to lowering cost as copolymers are more flexible and therefore expand the applications of PHAs currently. For increasing PHA production yield, investigating how PHA contributes to stress tolerance in bacteria can lead to improved strategies for cultivation of PHA-producing strains. Given that pilot-scale microbial community production of PHAs has only been done with wastewater inocula, these new strategies can enable us to use more microbial community sources, such as landfills,

in order to utilize waste feedstocks, such as dairy waste, and non-recyclable paper waste. This research will help contribute to building a circular bioeconomy, in which agricultural and forestry waste can be upcycled into a biodegradable product.

Animal Health Component

Animal Health Component: 0%

Research Effort Categories

Basic	50%
Applied	50%
Developmental	0%

Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
511	4099	1040	34%
133	0660	1040	33%
403	3470	2020	33%

Knowledge Area

[511] New and Improved Non-Food Products and Processes

[133] Pollution Prevention and Mitigation

[403] Waste Disposal, Recycling, and Reuse

Subject Of Investigation

[4099] Microorganisms, general/other

[0660] Paper and pulp derived products

[3470] Other dairy cattle products

Field Of Science

[1040] Molecular biology

[2020] Engineering

Keywords

biodegradable plastic, microbiology, stress response, bioproduction, Agricultural waste conversion

Goals / Objectives

Specific objectives: Currently, the cost of PHA production is too high for industrial application, and there is limited versatility in PHAs produced. At the same time, we are learning more about the natural roles that PHAs play in protecting bacteria from environmental stressors. I propose utilizing this emerging knowledge to innovate PHA

production strategies from agricultural waste. My objective is to: 1) produce PHB-co-PLA from a mixture of dairy and paper waste, 2) improve PHA and co-polymer yields from single strains by utilizing secondary stressors, and 3) enrich PHA producing microbial communities from various environmental sources through utilization of secondary stressors.

Project Methods

APPROACH- Objective 1: Produce PHB-co-PLA from a mixture of dairy and paper waste: Preliminary data: Preliminary results show production of PHB-co-PLA from synthetic acid whey (M9 minimal media + 5 g/L lactate + 37.5 g/L lactose). The gene for propionyl-coA transferase (pct), derived from Megasphaera elsdenii, was cloned into our existing PHA-production plasmid, pBBR C1ABJ4 using Gibson assembly. Following verification of assembly, the resulting pct-containing plasmid (pBBR C1ABJ4-Pct) was transformed into E. coli LSBJ, a strain with mutations that serve to divert carbon flux towards PHA production. E. coli LSBJ + pBBR C1ABJ4-Pct was inoculated into 100 mLs synthetic acid whey in triplicate, and demonstrated production of PHB and PLA. Polymer purification will be performed by the Kumar lab for final confirmation of polymer structure.To build upon these preliminary results, I will grow E. coli LSBJ + pBBR C1ABJ4-Pct on real acid whey (AW) as well as AWWF, as produced by our collaborators at the Kumar lab at SUNY-ESF. After growing E. coli LSBJ + pBBR C1ABJ4-Pct and E. coli LSBJ + pBBR C1ABJ4 (control strain) on both wastes, I will lyophilize the cells, perform a PHA extraction protocol previously used in our lab [3], and measure thePHB and PLA content and yield using gas chromatography with flame ionization detection (GC-FID). Expected outcomes: I expect PHB-co-PLA to be produced from real acid whey, and AWWF mixture, as we have previously shown that using synthetic acid whey media containing some of the sugar components of these wastes (glucose, lactose, lactic acid) results in PHB-co-PLA production. I expect there to be differences in quantity of co-polymer produced, perhaps inhibition of production coming from real wastes given that they contain potentially inhibitive compounds (i.e. acetic acid) [6]. Potential pitfalls: If no PHB-co-PLA is produced from these wastes, I will investigate what is inhibiting this production by testing various dilutions of these wastes to find the threshold at which co-polymer production begins to decrease. I will also use high performance liquid chromatography (HPLC) to check for any accumulation of inhibitory compounds and compare those levels with that of synthetic paper acid whey mixture (SyntheticPA) previously developed in the lab.Objective 2: Improve PHA and co-polymer yields from single strains by utilizing secondary stressors: Objective 2.1: Investigate effects of oxidative stress on PHA production and activity of enzymes relevant to PHA production. I will set up 100mL cultures in triplicate in M9 + 20 g/L glucose and SyntheticPA containing the highest sublethal concentration of H_2O_2 (oxidative stressor) for each strain, and incubate for 48 hours (30°C, 200 rpm). Then I will take samples for quantifying enzyme activity for enzymes relevant for PHA production and the TCA cycle. I will harvest cells, then extract PHA using a previously established protocol, and perform GC-FID to measure PHB content. Expected outcomes: In cultures with sublethal amounts of H_2O_2 added, I expect to see increased enzyme activity of NADPH-producing enzymes and PhaB, as well as increased PHB yield. Objective 2.2: Investigate effects of hyperosmotic stress on PHA depolymerase enzyme activity, cell structure, and PHA production: I will set up 100 mL cultures in triplicate in M9 + 20 g/L glucose and SyntheticPA containing the highest sublethal concentration of NaCl (hyperosmotic stressor) for each strain, and incubate for 48 hours (30°C, 200 rpm). Then I will take aliquots out for quantification enzyme activity as described previously for PhaB and PHA depolymerase. I will harvest the cells, extract PHA, and perform GC-FID to measure PHA content. Expected outcomes: In cultures with sublethal amounts of NaCl added, I expect to see increased enzyme activity of PhaB, decreased enzyme activity of PHA depolymerase as well as increased PHB yield. I will also perform transmission electron microscopy (TEM) to look at changes in PHA granule size, coalescence, and cell structure as previous literature has described. Potential pitfalls and alternative approaches: If I do not see expected results from Objectives 2.1 or 2.2, I will test stationary phase application of these stressors, as well as dual initial and stationary phase application, since these conditions resulted in increased PHB yield from C. necator in a previous study. If applying neither oxidative nor osmotic stress result in increased PHB production, I will utilize toxins as a stressor; adding levulinic acid to culture media has resulted in increased PHB-co-PHV production from C. necator. As paper waste is a part of my mixed waste feedstock, lignocellulosic toxins like levulinic acid or furfural are relevant in this context.Objective 3: Novel enrichment for PHA production from environmental inocula, using osmotic stress: Preliminary data: Previous research in our lab involves analyzing and enriching landfill microbial communities for the purpose of plastic degradation. Soil samples from the

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Dane County Landfill in Madison, Wisconsin were collected and then enriched on minimal media containing a mix of petrochemical plastics. These communities were sequenced using 16S rRNA sequencing and I analyzed the resulting OTUs to determine the fraction ofpotential PHA producing-OTUs in each respective landfill-derived community enriched on that mix of plastics. I identified four communities containing various proportions of PHA producers to test for PHA production. To build upon my preliminary data, I will test an adapted version of a previously established osmotic selection regime on these 4 landfill inocula. These communities will be inoculated into M9 + 20 g/L glucose or SyntheticPA media and undergo that modified osmotic enrichment regime. For all communities, I will take four timepoints to perform DNA and RNA extraction, and quantify PHA at timepoints 1 and 4. All DNA samples will be prepared for 16S rRNA sequencing. I will again quantify the proportion of potential PHA producers in each community at each timepoint. After seeing which landfill community yields the most PHA, I will perform RNAseq on those samples to examine changes in regulatory genes that control PHA production. Expected outcomes: I expect a slight increase in potential PHA producers at time-point 1 compared to original community composition, and a larger increase at time-point 4. Future experiments that are more focused on increasing PHA yield from a promising microbial community will include longer culture periods. Potential Pitfalls: If I am unable to enrich PHA production, I will try other strategies from previous literature that incorporate different stressors such as oxidative stress or pH stress. Alternatively, I could investigate effects of under-studied nutrients on PHA production. For example, little is known about the role of, type, and level of sulfur containing compounds in PHA production, even though it is an essential element for the TCA cycle, which feeds into PHA production.