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Bacillus megaterium & Papiliotrema laurentii: Competing roles in polymer coating degradation?

Honors Thesis

Anna Blumberg

Department: Chemistry

Advisor: Justin Biffinger, Ph.D.

April 2023

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Abstract

The design of coating formulations that have material properties that can hinder the biodegradation of a protective surface without aggressive or environmentally harmful additives will require a detailed understanding of knowledge gaps at the biotic-abiotic interface involving a variety of microorganisms. This project focused on two isolates extracted from a biofilm which was found to degrade Air Force cargo aircraft topcoats. These organisms, *Bacillus megaterium* (gram-negative bacterium, non-hydrolytic but alkane oxidizing) and the hydrolytic yeast *Papiliotrema laurentii* were initially observed as individual cultures and then as a co-culture. The combination of these two organisms (under similar growth conditions) provided a glimpse into more complex biofilm dynamics and the synergies these organisms maintain during the degradation of polyester polyurethane and the more recalcitrant polyether polyurethane topcoat, AS P-108.

Acknowledgements

I would like to thank my advisor Dr. Biffinger for his guidance and support throughout my time in his lab. Also, my project would not have been possible without my AFRL and NRL collaborators. I extend a thanks to the University of Dayton Chemistry department.

I thank the Dean's Summer Fellowship program for funding my summer research as well as the UD Honors Program for their financial contribution to my thesis work. I also greatly appreciate my funding through SERDP(WP1381). I extend my gratitude to the US Army for awarding me the financial aid needed for me to attend the University of Dayton. Most importantly, I want to thank my family for their support as well as the late Betty White for being my greatest inspiration.

University *of* Dayton

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I. INTRODUCTION

Microbial growth on polymer-coated weapons systems often occurs in the form of consortia (mixtures of microorganisms) which are either adhered to the surface or are free-floating in an aqueous environment above the surface. A consortia which adheres to the surface is known as a biofilm [1]. Biofilm formation is often a microbial response to a stressful environment such as one deprived of adequate nutrients, high UV radiation, or the presence of antimicrobial agents [2]. Life within these biofilms is heterogeneous, dynamic, and reliant on a complex web of interactions between constituents [3]. The extracellular matrix (ECM) of these biofilms offer protection from microbial predation, biocidal agents, as well as other environmental threats [2]. Given the complexity of biofilm dynamics and the collective advantage to the consortia of microbes, there is debate as to whether biofilms are a mere collection of individual organisms or are in fact multicellular organisms [3]. Gaining further insight into the interactions and overall dynamics within these formations will grant the knowledge required to prevent their future growth and thus, future degradation.

The polymer coatings facing degradation by these biofilms are recalcitrant with non-hydrolysable linkages. Despite their hardiness, the polymers are gradually degraded due to a combination of both environmental and microbial factors [4]. These plastics, being polyurethane polyethers, are designed for resistance to breakdown but are failing to fulfill this purpose. There are two paths forward in the development of more resistant polymer coatings. More aggressive, environmentally harmful metal-based compounds such as chromate could be added to ward off unwanted microbial life or a carbon based polymer which lulls microbes into a state of quiescence (dormancy) rather than cell death. Cell death is an unfavorable conclusion as it would lead to the release of all cellular contents and possibly accelerate polymer degradation. An environmentally harmful coating could pose a threat to human life, such as the servicemen and women working on the aircraft, and would almost certainly select for more harmful, hardy microorganisms to survive. This would lead to a dilemma analogous to that of antibiotic resistance.

In addition to posing a threat to aircraft polymer coatings, this glimpse into biofilm mediated polymer degradation could be a step forward into the controlled biodegradation of plastic wastes. The increasing use of polyethylenes (PE) has contributed to an accumulation of plastic waste in the environment. The use of biofilms capable of biodegradation of these compounds would provide a cheap, effective solution to this worsening issue [5]. Biofilms known to degrade plastics, such as those degrading the observed airplane coatings, could be harnessed for good, but must first be understood and studied.

In collaboration with Air Force Research Laboratory scientists, consortia of microorganisms were isolated from areas of polymer degradation on Air Force cargo aircraft. The consortia were screened for hydrolytic activity on the colloidal polymer, Impranil and organisms which tested positive were isolated and identified. Of these hydrolytic organisms, *Papiliotrema laurentii* was identified as a prolific degrader of polyesters. This non-motile yeast was able to secrete enzymes out into its local environment in order to degrade the surrounding coating. While it may degrade polyesters, *P. laurentii* is not able to immediately degrade polyether coatings, such as the original aircraft coating, as these compounds are not readily hydrolysable. A bacterium from the original consortia, identified as *Bacillus megaterium*, is a known alkane oxidizing organism and was hypothesized to play a role in converting ethers to esters in order to facilitate polyester hydrolysis.

Bacillus megaterium is a gram positive soil bacteria known for industrial practicality. It is known to produce a wide variety of enzymes which allow it to be used for a number of synthetic reactions [6], some of which are rarely observed in nature such as oxidations of silicon containing compounds [7]. Its ability to carry a number of plasmids, which are extrachromosomal DNA strands, allows for a great deal of genetic diversity and adaptability. *B. megaterium*'s ability to produce esterases as well as its potential to produce a variety of other enzymes indicate it is potentially an important player in the observed biodegradation. *P. laurentii* is also an organism known for its industrial practicality. Its secretion of a range of enzymes, especially lipolytic enzymes, indicates significant usefulness [8]. It is essential to determine the dynamics between these two organisms so as to develop a piece of the bigger picture of biodegradation.

II. METHODS

The general experimental framework was a bottom-up approach in an effort to piece together the greater consortia dynamics. In order to achieve these ends, single cultures were isolated and tested to preface the co-culture testing of the same nature. **Figure 1** summarizes this approach as well as indicates there is simultaneous work to be done outside of this project regarding other isolates from the consortia. Ultimately, the aim of this project was to elucidate the dynamics of the two isolates and determine whether this particular co-culture plays a role in environmental biodegradation.

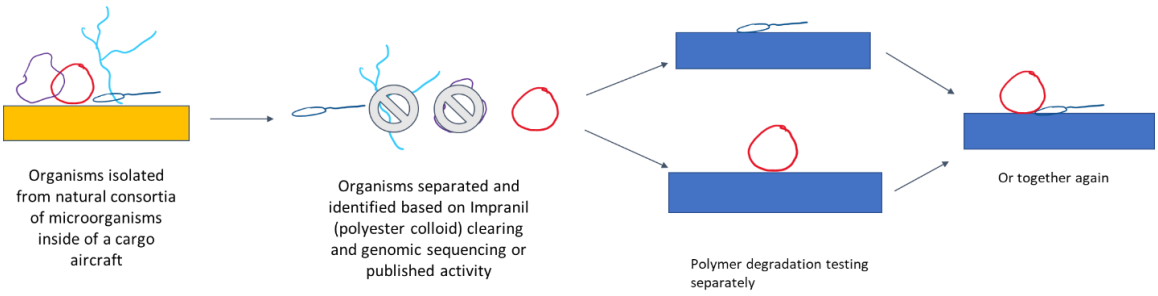


Figure 1 General framework for bottom-up approach to establishing co-culture interactions

Initial Viability Testing and Hydrolytic Screening

To establish baseline information, collaborators at AF Wright Patterson base analyzed the cell viability of cultures grown in yeast nitrogen broth (YNB) over a 7 day period. This was done using a FUN- fluorescence viability stain and a Baclight bacterial viability stain for the *P. laurentii* and *B. megaterium* cultures respectively. The percent viability was measured using standards of different proportions of live and dead cells as well as by recording the fluorescence emissions at standard wavelengths. The initial intent for the growth experiments was to create conditions under which the bacterium and fungus would grow in the same media and give the same response when drop cast on a polymer coated surface. In nutrient rich conditions, *B. megaterium* is able to grow at approximately twice the rate of *P. laurentii*. **Figure 2** indicates that without additional carbon sources, *B. megaterium* displays more rapid growth than *P. laurentii*. Future

research may include additional amino acid supplementation to better define the minimal medium without complex carbon source additives in order to control *B. megaterium* growth prior to experimentation.

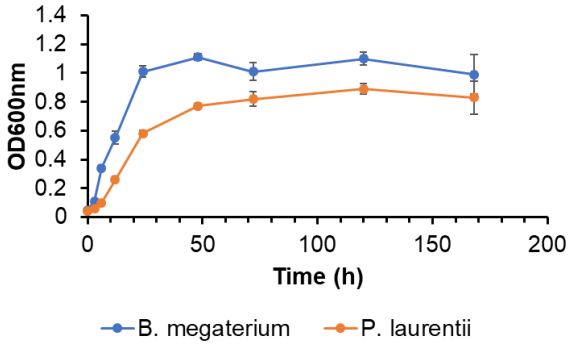


Figure 2 Growth comparison of *B. megaterium* and *P. laurentii* in yeast nitrogen broth

The results of the cell viability give insights into the rate of growth of each organism in a nutrient deprived, nutrient poor, and nutrient rich media. Figure **3** results show cell viability for *P. laurentii* (A) and *B. megaterium* (B). Generally, *P. laurentii* showed higher cell viability over the 7-day period in nutrient rich (succinate) media than *B. megaterium*. *B. megaterium* showed a rapid growth and reduction in viability over the first 24 hours in all 3 media, though this viability stabilized at around 20-25% after 24 hours in both the nutrient poor and nutrient rich conditions. These observations are similar to an adaptation to salt stress noted in another strain of *B. megaterium* [9]. P. *laurentii* cell viability decreased with relative stability over the 7 day period with the nutrient rich condition being the favorable medium.

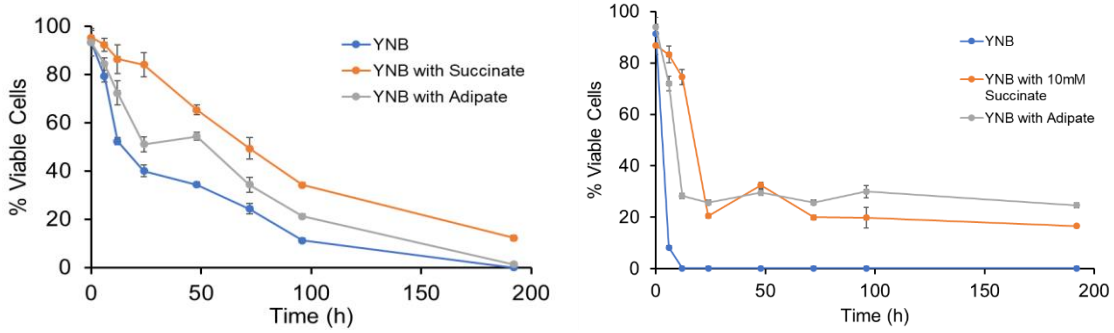


Figure 3 Viability of (A) *P.7-dayentii* and (B) *B. megaterium* over 7 days beginning at early stationary phase

From these viability results, *B. megaterium* and *P. laurentii* were qualitatively screened for hydrolytic activity. Given the established growth patterns of each organism, comparable cell densities of the bacterium and fungus were drop cast while in the early stationary phase. Both organisms were grown in liquid medium and plated on tryptic soy agar with 1% Impranil, a hydrolysable polyester polyurethane colloid [10]. The dark ring surrounding the fungal colonies is an indication of Impranil degradation and the lack of clearing around *B. megaterium* is an indication that it is not capable of the hydrolysis of polyesters. The raised structure taken on by *B. megaterium* is an abnormal one and is a typical starvation response in the family *Bacillus*. This structure may be an indication that the bacterium is entering a state of sporulation to counter this starvation [11].

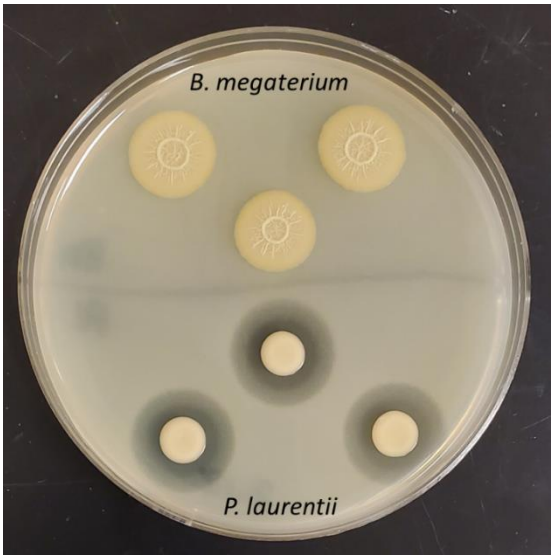


Figure 4 TSA plate with 1% Impranil (by weight) of *B. megaterium* and *P. laurentii*

In addition to the single cell colonies, a roughly 50/50 mixture of *B. megaterium* and *P. laurentii* (based on optical density values at 600nm) was drop cast on a TSA 1% Impranil plate. **Figure 5** shows the results of this mixture. It appears as though *B. megaterium* is physically isolating *P. laurentii*. These findings were unprecedented and indicate the relationship between the two organisms is likely not synergistic but is instead potentially antagonistic. The potential ongoing competition as well as the lack of

homogeneity throughout the mixed colony are results which give way to more questions than answers.

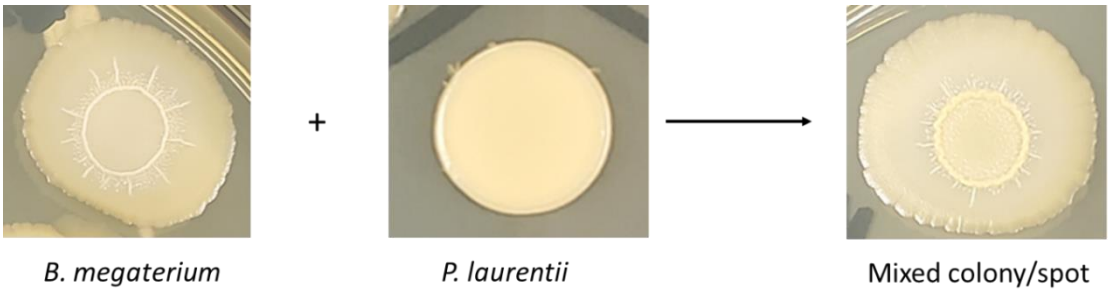


Figure 5 TSA plate with 1% Impranil (by weight) of a mixed colony

Culture conditions and supernatant concentration

The bacterial and fungal cultures were prepared from slants that had previously been inoculated using an original frozen isolate sample. The cultures were grown in their appropriate liquid media. *B. megaterium* was found to grow most efficiently in tryptic soy broth (TSB) at pH 7 while *P. laurentii* was grown in yeast mold broth at pH 4.

To prepare initial baseline secretome samples for analysis, the organisms were grown in single cell cultures and a co-culture in TSB 5 and TSB 7. Following a 7-day growth period, the supernatant from each sample was collected after centrifugation at 14k rpm for 1 min. These supernatant samples were syringe filtered (0.2µm polycarbonate filter) to remove any leftover cells. After flash freezing with liquid nitrogen, the supernatants were freeze dried overnight or until the samples were entirely dry. The freeze-dried product was then dissolved in 6mL sterile reverse osmosis (RO) water and placed in a centrifugal protein concentrator with a molecular weight cutoff at 10kDa before being centrifuged for 15 minutes at 6000 rpm. Following this, the samples were syringe filtered once more and then stored at -20°C.

Growth Experiment & Co-Culture

Following the baseline analyses, the same inoculation procedure was used to create cultures in yeast nitrogen broth pH 7 in order to simulate the low-nutrient environment of the original airplane coatings. In this growth experiment, samples were

supplemented with 20mM glucose (positive control), 20mM succinate, and 20mM adipate as well as keeping a negative control.

Each co-culture was prepared using an appropriately diluted sample of *B. megaterium* and *P. laurentii*. Optical densities were used to ensure a roughly 50/50 mixture of each organism was added to the co-culture. This would allow both organisms to begin with a roughly similar initial cell count and keep one organism from greatly outnumbering the other.

In each culture, the inoculation sample was 2% of the entire volume, with the entire volume being 50mL. A relatively large initial volume was used to ensure that the data collection throughout the experiment would have a minimal effect on results. By the conclusion of the growth experiment, less than a fourth of the prepared culture was collected. For the duration of the data collection period, the cultures were left in a gently shaking 27 C incubator.

Initially, collaborators at AF Wright Patterson base analyzed cell viability in YNB over a similar time period. This was done using a FUN- fluorescence viability stain and a Baclight bacterial viability stain for the *P. laurentii* and *B. megaterium* cultures respectively. The percent viability was measured using standards of different proportions of live and dead cells as well as by recording the fluorescence emissions at standard wavelengths.

The data collection samples were also used to measure the degradation of the carbon sources by the organisms over time. This was done using High-Performance Liquid Chromatography (HPLC) in order to accurately determine the concentration of the carbon source present in the culture and establish what, if any, degradation was taking place. HPLC was done using an organic acid analysis column (Hiplex-H', 7.7x300mm 8um, Agilent) and a refractive index detector. The mobile phase was run using 0.05M H_2SO_4 with a flow rate of 0.5mL/min at 60 C.

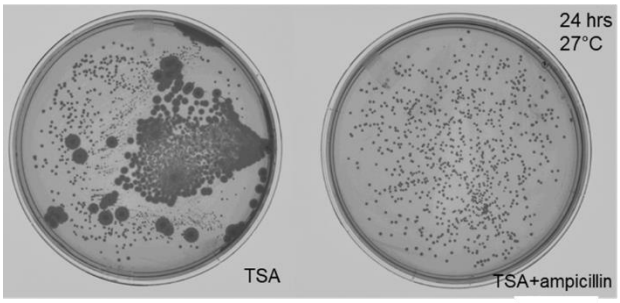
Plating

Throughout the growth experiment, supernatant was collected from each single organism culture and the co-culture for the purpose of analysis over time. Two 1mL samples were collected from each culture at 6 hours, 18 hours, 24 hours, 72 hours, and

144 hours post-inoculation. 1mL of each of these cultures was used to prepare tryptic soy agar plates to observe the survivability and growth of the organisms in the liquid media. The samples were diluted using typical serial dilution protocol in phosphate buffered saline before being plated in an aliquot of 100 uL.

The individual organism growth within the co-culture was of particular interest so antibiotic and antifungal plates were prepared using 100 uL/mL ampicillin and 100 ul/mL cycloheximide respectively. A number of antifungals and antibiotics were tested in order to find one which was able to fully dissolve in the tryptic soy agar after the agar underwent steam sterilization. This method of using selective plates allowed for the isolation of each organism from the co-culture as well as proved useful in minimizing contaminants amongst the single-culture plates. Selective plating allowed for the counting of viable colonies produced by each culture in liquid media. In order to elucidate the dynamics and relationship of the two organisms, their ability to grow and thrive in the co-culture needed to be determined. The change in the two populations over time granted insights into these interactions as well as established which media allowed for survival and which enabled the organisms to thrive. **Figure 6** shows the results of the established selective plating technique. The growth of *P. laurentii* appeared to be unaffected by the presence of the antibiotic ampicillin while the antibiotic prevented the growth of *B. megaterium*. The results of the antifungal plates showed similar results with *B. megaterium* growth as well as antifungal selectivity.

B. megaterium and *P. laurentii*



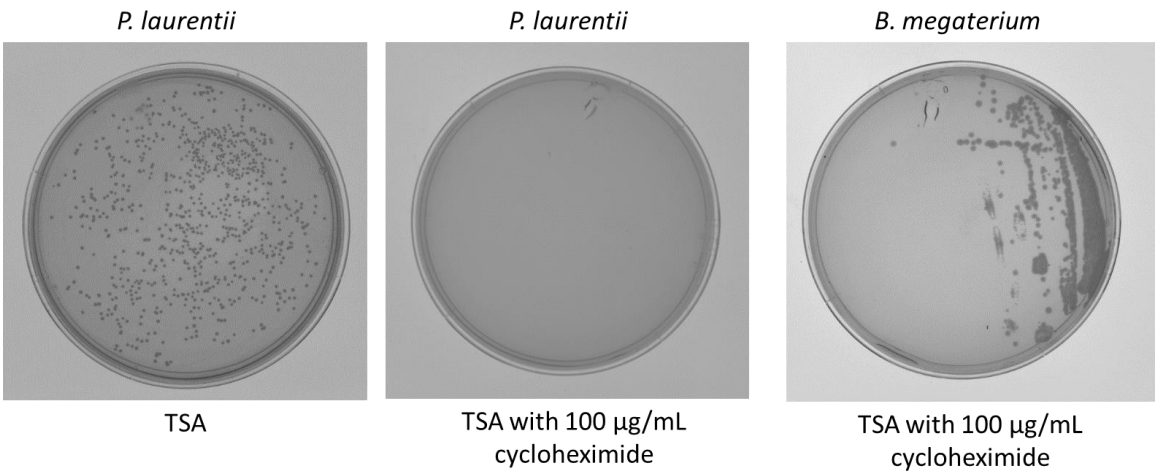


Figure 6 Selective plating technique using cycloheximide and ampicillin

Gel Electrophoresis & Staining

Gel electrophoresis was used to separate and analyze the protein secretome of each culture. Each gel contained up to but no more than 4 supernatant samples at a time to maximize stain quality. 1 mL of each data collection sample was prepared using the supernatant protocol (detailed above) and was then used for gel electrophoretic analysis. Novex Native gels were used to prepare the intact secreted proteins for further protein concentration and enzyme analysis. The samples were prepared using standard Novex protocol. A 4-20% tris glycine gel was used as the protein sizes could not be predicted prior to the analysis and the gel was run using pre-made running buffer in accordance with the Novex protocol. The gels were run at 225 volts for 30 minutes or until the protein ladder reached a designated endpoint.

Two staining procedures were used to analyze the supernatant samples. The silver staining procedure was used to analyze the relative protein concentrations of each sample. The silver staining protocol used was the standard Thermo Fisher procedure. The darkness of present bands was used to estimate the quantity of protein in the supernatant sample. The Zymogram or hydrolase procedure was used to stain any hydrolytic proteins a vibrant red color. Zymogram procedure began with a 30 minute 0.1M phosphate buffer wash. Given the temperature dependence of the test, the gel was stored in a shaking 25 C incubator between wash changes. The initial buffer wash was followed with a 30-minute Triton X 5% wash and then a second 30 minute 0.1M phosphate buffer wash. The gel

was then placed in a solution containing 90mL 50mM phosphate buffer with 10mL methanol (100 mL total) and 186mg naphthyl acetate for 15 minutes. This solution was heated and cooled to room temperature to fully dissolve any solids before being used as the next wash. Finally, the gel was moved to a buffer containing 100mL 50mM phosphate buffer and 25.7mg Nile red stain and left overnight.

Polymer Degradation

The growth and carbon degradation testing in liquid media was followed by testing the growth and degradation of dry polymer coatings. Polyethylene succinate and polyethylene adipate were drop cast onto their own respective microscope slides and given 24 hours to dry completely. Simultaneously, a *B. megaterium* culture and a *P. laurentii* culture were prepared from frozen biomass and grown for 2 days in a gently shaking 27 C incubator. These cultures were used to prepare TSA streak plates. Once distinct colonies formed, following a period of 2 days, plastic inoculation loops were used to extract 2 or 3 colonies. These colonies were added to 0.3mL PBS and then used to streak the polymer coated microscope slides in the fashion shown in **Figure 7**. For each polymer, there was a single organism slide for both the fungus and bacterium as well as a slide with both (one streaked vertically and the other streaked horizontally), and a slide with only PBS to act as a negative control. The slides were then stored in a 27 C incubator in 95% humidity for a period of 7 days.

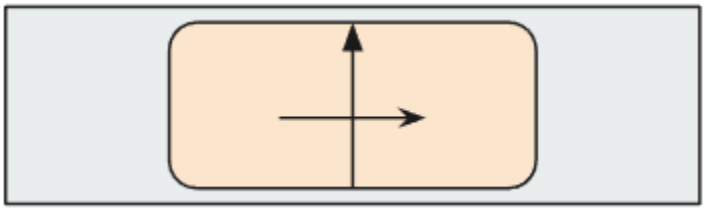
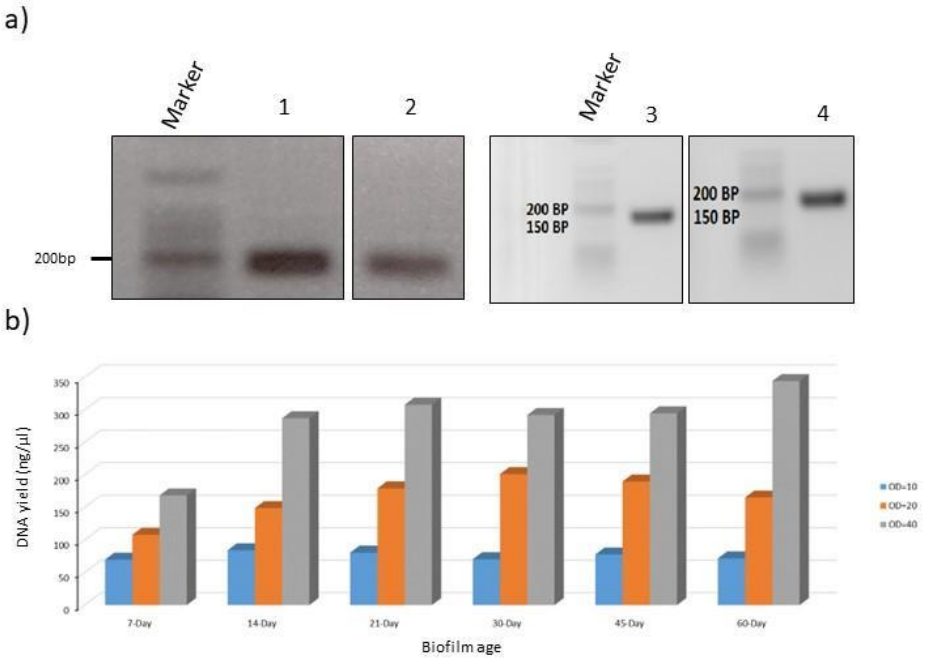


Figure 7 Streaking technique of polymer degradation on microscope slides

qPCR

PCR analysis was performed by Air Force collaborators at Wright Patterson Air Force Base as a way to determine the DNA yield of single cell cultures as well as a co-culture over a 60-day period on nutrient deprived fluorodishes. For this analysis, primers of the *recA* gene of *B. megaterium* and *P. laurentii* were designed to target the cutinase-

like gene. In each organism, these are single-copy genes. **Figure 3** shows the PCR amplicons against pure *B. megaterium* (lane 1), mixed DNA containing both organisms (lane 2 and 4), and pure *P. laurentii* DNA. These results validate the primer accuracy and show that the mixed DNA samples also produced an amplicon thus indicating there was no cross-reactivity. Increasing amounts of bacterial and fungal inoculum were used based on optical densities (600nm) in order to determine the minimal quantity needed to perform the PCR. The DNA yield was dependent on the quantity of inoculum used in the sample.



Caption: Validation of qPCR primers and DNA yields from co-culture biofilms composed of Bacillus megaterium and Papiliotrema laurentii.
a) DNA gel electrophoresis showing amplicons from indicated template samples: 1, recA gene amplicon using B. megaterium gDNA; 2, recA gene amplicon from co-culture gDNA extracts composed of B. megaterium **and** P. laurentii gDNA; 3, cutinase gene amplicon using P. laurentii gDNA; 4, cutinase gene amplicon from co-culture gDNA extracts composed of B. megaterium **and** P. laurentii. b) DNA yields of temporal DNA extractions from nutrient starved biofilms composed of B. megaterium and P. laurentii mixed at different OD600 ratios. B. megaterium and P. laurentii were mixed at 10, 20 or 40 OD600.

Figure 7 A) DNA gel electrophoresis displaying amplicons from template samples: 1, recA gene amplicon using *B. megaterium*; 2, recA gene amplicon from co-culture gDNA extracts; 3, cutinase gene amplicon using *P. laurentii* gDNA; 4, cutinase gene amplicon from co-culture gDNA; B) DNA yields from nutrient deprived co-culture biofilms at varied OD_{600} values

III. RESULTS & DISCUSSION

Growth Experiment & Co-culture

In addition to initial cell viability testing, the ability of the organisms to degrade given carbon sources was analyzed to give a multi-dimensional look into the microbial activity. While both organisms were able to degrade and utilize succinate as a viable carbon source, adipate proved to be a poor, unusable carbon source.

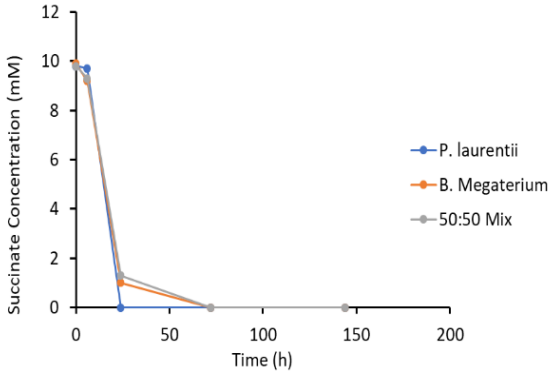


Figure 8 A) Organic acid analysis using HPLC, Succinate

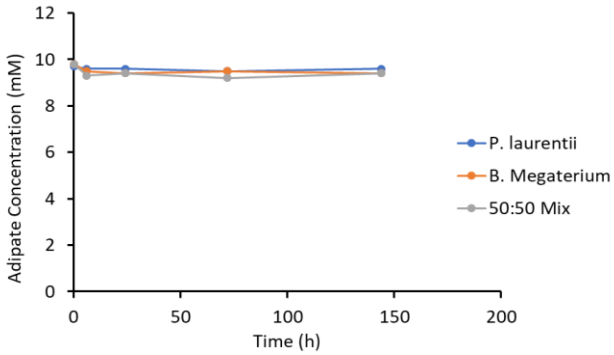


Figure 8 B) Organic acid analysis using HPLC, Adipate

Plating

The purpose of plating growth experiment samples was to determine the growth pattern of each organism in a co-culture over a period of time. This was done by manually counting colony forming units (CFUs) as well as measuring the OD_{600} value of each sample. While CFUs correlate directly with the growth of the organism, optical density values measure both living and dead cells as well as cellular debris.

As shown in **Figures 9** and **10**, in the co-culture, both organisms grew rapidly over the first 24 hours as seen in the initial increase in CFUs. This pattern is also seen in the OD_{600} values for both organisms as well as the co-culture. After the first 24 hours, *P. laurentii* was shown to decrease growth and CFUs were too low to be detected by hour 144. *B. megaterium* experienced a peak in growth at 72 hours and then showed the potential beginning of a slow decline. Optical densities for all three samples began to decrease steadily after 24 hours. These results indicate that *B. megaterium*, given a carbon rich source, is able to reach a population peak and maintain growth for at least 144 hours. Conversely, *P. laurentii* cannot maintain its population given an abundance of nutrients past the initial 72 hours.

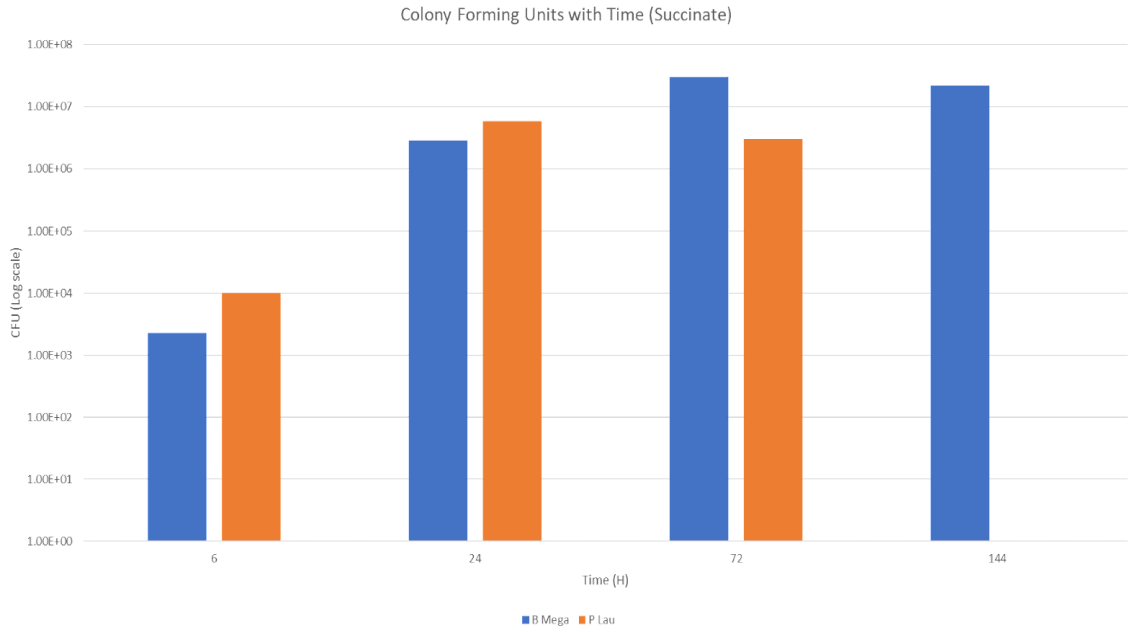


Figure 9 CFUs (log scale) of *B. megaterium* and *P. laurentii* grown in succinate over 144 hours

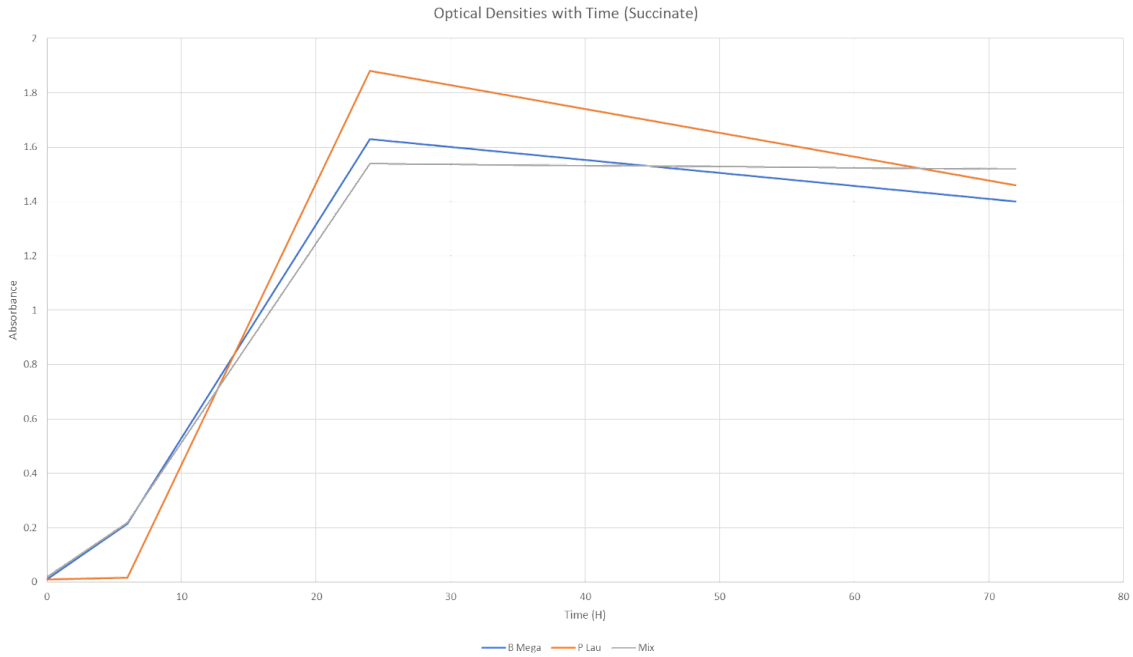


Figure 10 OD_{600} values of *B. megaterium*, *P. laurentii*, and co-culture over 144 hours

The results shown in **Figures 11** and **12** indicate both organisms in co-culture adopted significantly different growth patterns when placed in an adipate-supplemented medium. Given this carbon-poor source, *P. laurentii* reached peak growth at 18 hours and maintained a similar population for the rest of the experiment duration. *B. megaterium* showed considerable growth in the first 6 hours, became virtually undetectable between 18 and 24 hours, and peaked at 72 hours. Similarly to *P. laurentii*, *B. megaterium* maintained a consistent population following its peak. The peak CFU values for both organisms were significantly lower than in succinate, with a difference of between 10^2 and 10^3. Throughout the experiment duration, *P. laurentii* showed relatively low OD_{600} values. The peak in *B. megaterium* OD_{600} correlates with the peak seen in CFUs at 72 hours, indicating the results are consistent with sudden rapid growth at between 24 and 72 hours. The co-culture showed a noticeable increase in optical density between 24 and 72 hours as well, though this increase was not sustained through 144 hours. Ultimately, the results offer insights into the behavior of the organisms in nutrient-poor conditions where they cannot utilize the given carbon source. Under duress, both *B. megaterium* and *P. laurentii* appear to plateau their growth and endure the hardship but not thrive.

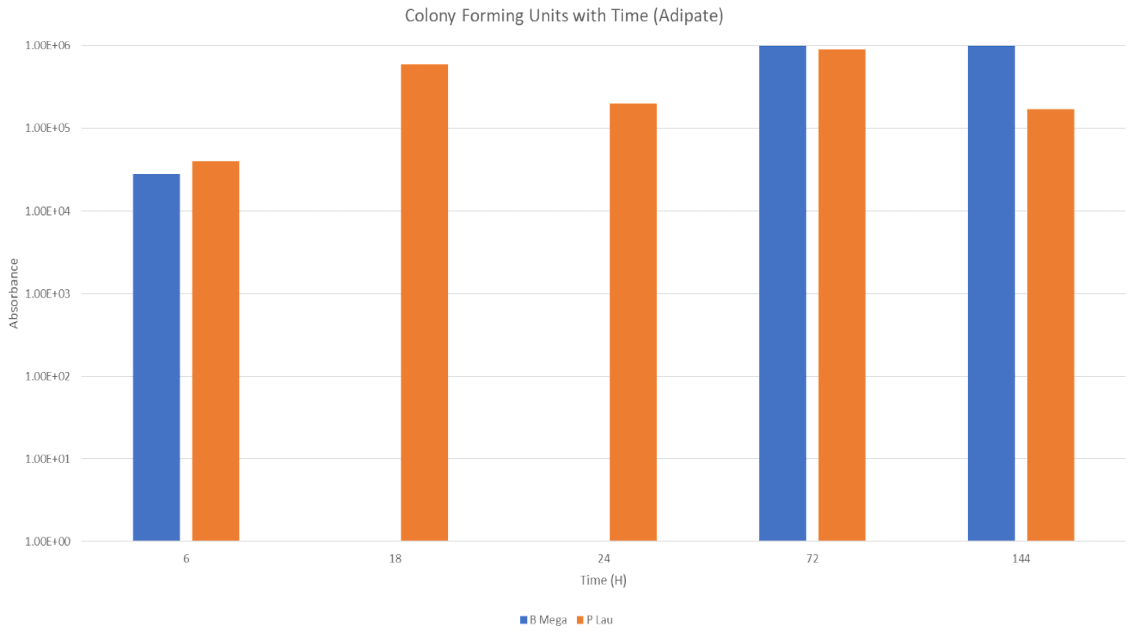


Figure 11 CFUs (log scale) of *B. megaterium* and *P. laurentii* grown in adipate over 144 hours

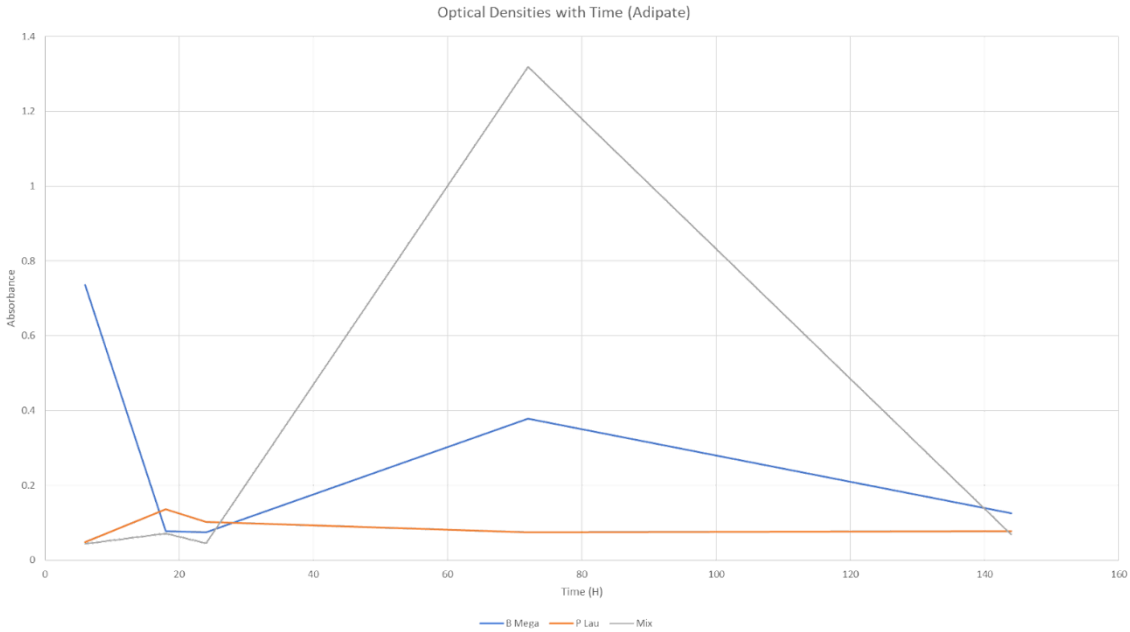


Figure 12 OD_{600} values of *B. megaterium*, *P. laurentii*, and co-culture over 144 hours

Gel Electrophoresis

The silver stain procedure produced qualitative data on the relative protein concentrations of each culture by protein size. The samples used for this analysis were

the 24- and 72-hour time points of each single organism culture as well as the mixture in both succinate and adipate. In **Figure 13A**, the results of the succinate mixture culture (SM) and succinate *B. megaterium* culture (SB) are shown. In the well nearest to the succinate *B. megaterium* sample (SB24), the positive control is shown to have fully developed, ensuring that the samples ran properly and that the stain is valid. The results of all other cultures were barely visible to the naked eye while the positive indicated stain success thus, indicating the protein concentration was too low to obtain valid results. The protein concentrations were highest at the 72-hour time point in both the mixture and *B. megaterium* samples with a number of proteins in each sample appearing to have the same weight.

The in-gel esterase, or Zymogram, produced qualitative results of hydrolytic activity by enzymes secreted into the supernatant. **Figure 13B**, which analyzed the same cultures as in **Figure 13A**, indicated hydrolytic activity in both the mixture as well as the *B. megaterium* sample. Despite *B. megaterium*'s ability to hydrolyze large polymers, it has been observed to hydrolyze much smaller compounds using lipases or esterases [12]. These results give insight into which proteins must be cut out and sequenced to determine the nature and identity of hydrolytic enzymes produced.

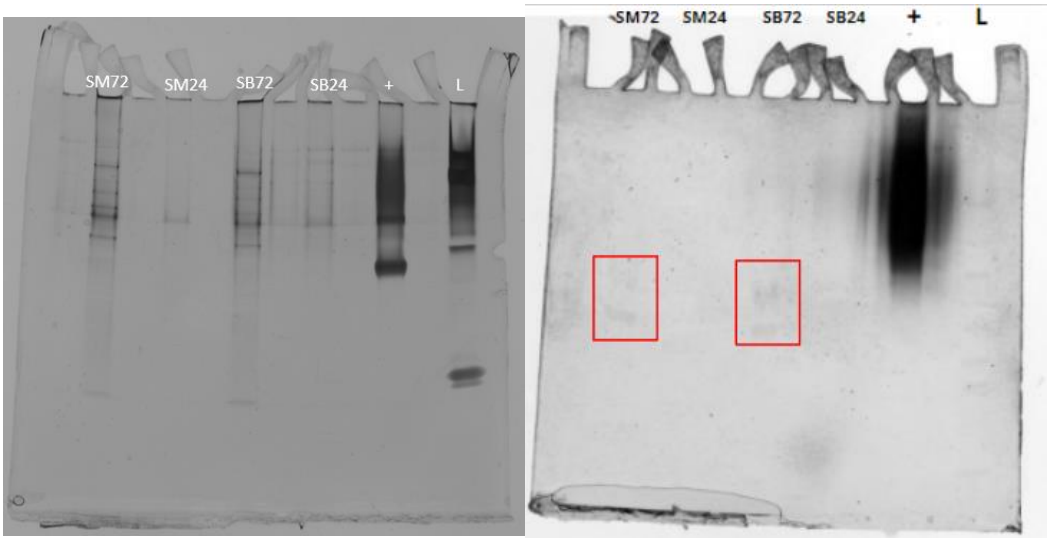


Figure 13 A) Silver-stained Native gel with samples Succinate Mix 72 hours, Succinate Mix 24 hours, Succinate *B. megaterium* 72 hours, Succinate *B. megaterium* 24 hours. B) Zymogram stained Native gel with the same schematic as A).

Polymer Degradation

Microscopy was used to produce qualitative observations of biodegradation by *B. megaterium* and *P. laurentii*. As seen in **Figure 14A**, the PES coating showed evidence of degradation by *P. laurentii* and the co-culture but no degradation by *B. megaterium* alone. This result conflicts with the above HPLC results as *B. megaterium* was shown to break down succinate in liquid medium. This conflict will require future experimental replication to rectify results. In **Figure 14B**, it is shown that PEA was not significantly degraded by either organism or the co-culture. However, *P. laurentii* may have had the ability to degrade the polymer at the site of colonies as evidenced by the spotted appearance around the initial streak. This may also be a result of cell death around the site of original streaking as the release of cellular contents could contribute to degradation.

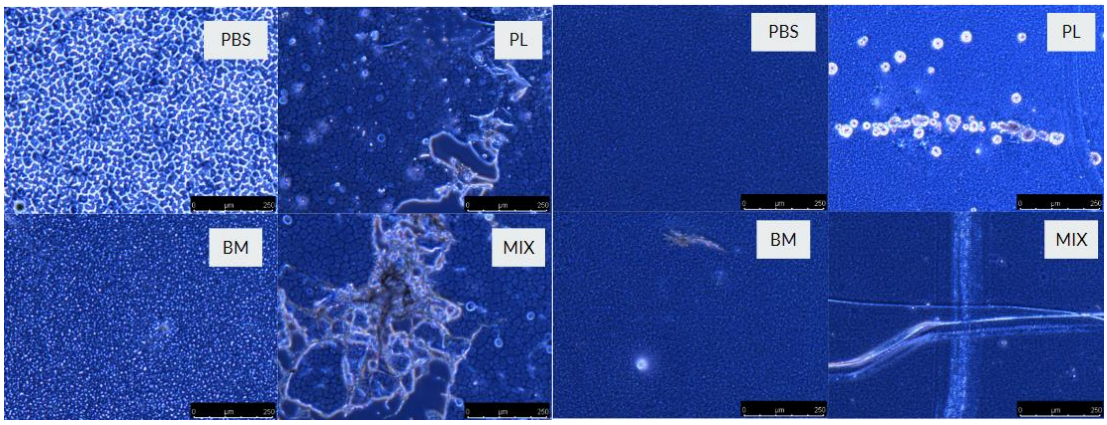


Figure 14 A) Polyethylene succinate (left) and B) polyethylene adipate (right) coated microscope slides

qPCR

The dynamics of DNA replication in a *B. megaterium* and *P. laurentii* biofilm are shown in **Figure 15**. Using OD_{600} values, the two organisms were mixed at a 1:1 ratio and placed on polymer-coated dishes. qPCR was done in triplicates for each of the biofilms. A standard curve was established using C_t values from qPCR experiments done by testing known DNA quantities and relationships to C_t measurements. On PES, both organisms experienced little change in DNA replication. On Irogan, PEA, and ASP-108, both the fungus and bacterium showed decreased replication initially and then peaked on

day 14. This could be an indication that these polymers are more resistant to biodegradation and thus are not viable carbon sources for growth.

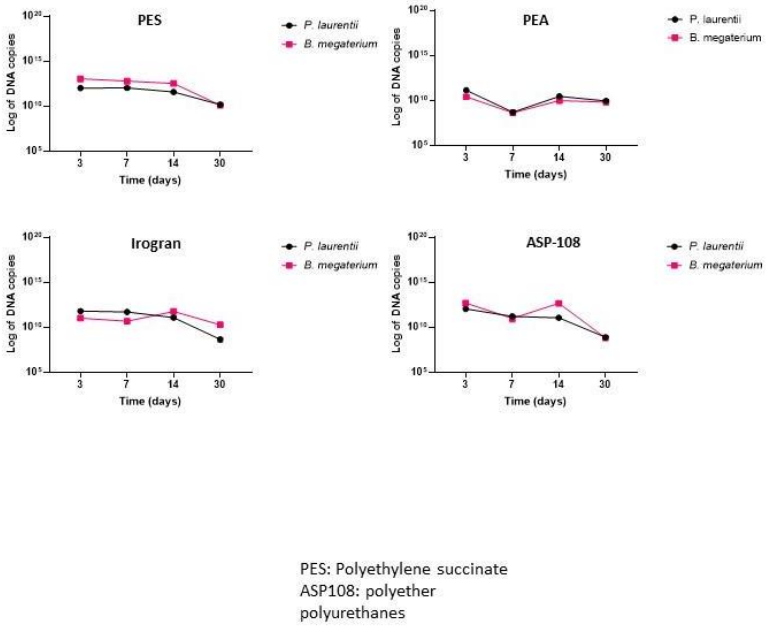


Figure 15 Genomic dynamics of co-cultures composed of *B. megaterium* and *P. laurentii* on polymer-coated fluorodishes. The genes recA (for *B. megaterium*) and cutinase (for *P. laurentii*) were targeted for quantitative PCR analysis to track DNA replication in the biofilms over time.

IV. CONCLUSIONS

My research project established a number of baselines as well as methods to evaluate biofilm dynamics in biofilms with two microorganisms. Throughout this project, a standard selective agar plate assay was established as well as a PCR based technique to monitor the populations of each organism within a co-culture over time. These results revealed that in nutrient-deprived conditions, *B. megaterium* is capable of surviving for weeks, but given a nutrient-abundant environment, it does not maintain viability past 7 days. These results also indicated a non-synergistic relationship between the fungus and bacterium which provides valuable insights into their dynamics within as a biofilm. Though neither *B. megaterium* nor *P. laurentii* were capable of independently degrading

recalcitrant coatings such as ASP-108, when combined as a biofilm there was clear indication that the greater consortia was capable of degradation. As of now, it has not been determined that this combination is responsible for the original degradation, but instead likely depends on other organisms within the consortia. Future research will build off of these foundational findings and utilize the techniques established so as to further peel back the layers of these complex biofilm dynamics. This work will expand on and replicate the experiments described above, especially growth experiments and polymer degradation observation via microscopy, in order to further determine the roles of *B. megaterium* and *P. laurentii* in the original degrading biofilm.

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