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Isolating and characterizing lipid degrading microbes from human decomposition soils

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Isolating and characterizing lipid degrading microbes from human decomposition soils

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Abstract

The compounds present in an environment shape the microbial communities that are found there. These microbes demonstrate diverse cellular activity at human decomposition sites. Lipase is an enzyme that is responsible for metabolizing lipids, and previous research has shown that lipase activity of bacteria increased in the soil below decomposing swine carcasses. In humans, a high body mass index (BMI) would typically mean more lipids, and thus, more lipase activity by microbes in soil. However, little is known about the relationship between microbial lipase activity and human decomposition. It is unknown whether BMI in human decomposition plays an important role in structuring the lipase activity of microorganisms in the soil. The purpose of this study was to isolate and quantify lipid degrading microbes in soils beneath decomposing human donors to understand how lipase activity in decomposition soils is affected by donor BMI. Soil samples were taken at various time points (measured as accumulated degree hours) beneath six decomposing human donors and control sites (> 1m from body) at the University of Tennessee Anthropological Research Facility, for a total of sixty-four samples. Diluted soil samples were inoculated on tributyrin agar plates, incubated, and evaluated for clearing zones around colonies. Donors were sorted into groups based on the parameters of BMI (healthy: 18.5 – 24.9, obese: > 30). Results indicated that the presence of a donor increases lipase

activity in soil; however, a higher BMI does not significantly affect lipase activity. These findings can provide insight into the microbial processes responsible for incorporating organic material into ecosystems.

Introduction

Microbial decomposition plays an important role in structuring and regulating soil ecosystems. Microbes are known to demonstrate diverse cellular activity in the soil, which is affected by the substrates available and environmental conditions. The physiochemical properties of soil can cause microbes to adapt rapidly (Lee et al. 2021).

One important factor that may affect microbial activity is the presence of lipids in the soil, which likely influence the production of lipase by bacteria. Lipase is an extracellular enzyme that is responsible for metabolizing lipids, which is accomplished by catalyzing hydrolysis of oils and fats (Rai et al. 2014). Research has indicated that lipolytic enzymes from microorganisms are more abundant in nature than those from plants and animals (Ramnath et al. 2017). This suggests that microorganisms are important to lipolytic processes in soil ecosystems and may be structured based on lipid availability.

Lipids and proteins are the primary components released into the soil during carcass decomposition (Howard et al. 2010). During decomposition, nutrient-rich fluids from the carcass are expelled into the surrounding soil, altering soil chemistry and microbial activity (Mason et al. 2022). Howard et. al (2010) showed that lipase activity of bacteria increased in the soil below decomposing swine carcasses. However, little research has focused on understanding how lipid inputs from decomposing human carcasses alters microbial activity in the soil.

In humans, a high body mass index (BMI) would typically mean more lipids, and thus, potentially more activity of lipase producing microorganisms in the soil. Differences in lipid

availability due to donor BMI may lead to differences in the abundance of microbes capable of lipase activity. It is currently unknown whether BMI in human decomposition plays an important role in structuring the lipase activity of microorganisms in the soil.

The purpose of this study is to isolate and quantify lipid degrading microbes in soils beneath decomposing human donors. Additionally, this study aims to understand how lipase activity in decomposition soils is affected by relative proportions of body fat using BMI. Due to substrate concentration, I predict that soils found below donors with an obese BMI will contain bacterial communities with increased levels of enzymes for metabolizing lipids compared to donors with a healthy BMI.

Methods

Study design. A total of sixty-four soil cores (5 cm) were obtained beneath six decomposing human donors (Table 1) and from 1 m away as a control at the University of Tennessee Anthropological Research Facility (ARF), located in Knoxville, TN. Soil samples were taken prior to decomposition and thereafter at various accumulated degree hour (ADH) intervals - until fluids were no longer leaking from the abdomen. Sampling for all donors in this study occurred during the summer (June- August). Cadaver height and weight were used to determine BMI. Six donors were selected for two experimental categories: healthy (n=3; BMI 18.5 - 24.9) and obese (n=3; BMI > 30), referred to as BMI codes.

For each soil sample, 1 g of soil was added to 9 mL of 0.85% filter sterilized NaCl (30 µ filter). The soil-NaCl solutions were then vortexed for 1 minute and diluted to a series of 10^{-2}, 10^{-3}, and 10^{-4} in fresh 0.85% NaCl. 100 µL of each dilution was transferred to a tributyrin agar plate (1% Tributyrin, 0.5% Peptone, 0.3% Yeast Extract, 2% Agar; pH = 7.00) using the spread plate method, yielding final plate dilutions of 10^{-3}, 10^{-4}, and 10^{-5}. Three replicates were

performed for each sample and dilution. Plates were incubated at 25°C for 72 hours and were then evaluated for colonies capable of lipase activity, denoted by clearing zones surrounding the colony (Figure 1).

Statistical Analysis. To account for slight differences between weights of soil used for each soil suspension, lipase colony counts were normalized to gram soil using the following equation: lipase CFU/g of soil (lipase CFU/ grams of soil plated). Additionally, the relative abundance of lipase colonies was calculated ((lipase colonies / total colonies) x100) to account for natural variation in the soil and total colony counts. Lipase colony forming units were analyzed before and after normalization of the colony counts relative to respective control samples. Prior to normalization, colony counts are expressed as lipase colonies per gram of soil. After normalization, they are expressed as relative abundance of lipase colonies per gram of soil. Lipase colony counts (CFU/g soil) and relative abundance of lipase colonies were used to test for significant difference between donor and control soil samples using two-sample t-tests. This was performed on all donors combined and each donor individually. All statistical models were analyzed in RStudio using the statistical packages (tidyverse version 1.3.2; ggplot2 version 3.4.0).

The variation in lipase colonies due to time (as ADH), BMI code (healthy or obese), and the interaction between the two factors was assessed with a hierarchical linear mixed-effects model, allowing for random slopes and/or intercepts by donor, which was chosen based on best fit (determined by Akaike information criterion [AIC]). The normalized CFUs and ADH were logarithmically scaled to run the linear mixed effects model. Linear mixed-effects models were run in R using the lmer() function (R package lme4 version 1.1.25) and then statistically assessed using ANOVA.

Results

Donor BMI varied between 22.00 and 54.10 for six the donors studied, and all other metadata can be found in Table 1. Results indicated that the mean lipase colony forming units (CFU) in soils below donors was significantly higher (mean = 4.69×10^7) compared to controls (mean = 2.86×10^6) ($p < 0.001$) (Figure 2). Further, the mean lipase CFU were significantly higher in decomposition soils below TOX008 ($p = 0.006$) and TOX010 ($p = 0.006$; Table 2) compared to other individual donors. For TOX008, TOX010, and TOX011 colonies capable of lipase activity in decomposition soils was highest between 0 and 3000 ADH, after which, lipase activity declined (Figure 3).

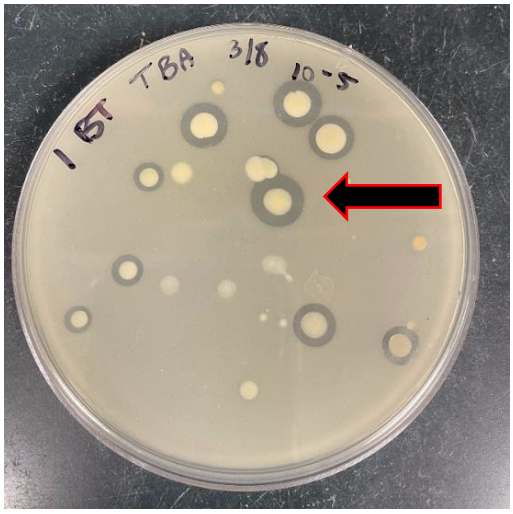

Figure 1. Colonies with and without clearing zones on a plate. An example of a colony with a clearing zone is denoted by an arrow.

Table 1. Metadata for each donor in the study

Donor	Weight (lbs)	Height (cm)	BMI	BMI Code	Age	Gender
TOX008	137	160	24.3	healthy	40	M
TOX009	131	158	23.8	healthy	72	F
TOX010	374	177	54.1	obese	65	M
TOX011	140	170	22	healthy	81	M
TOX013	278	174	41.6	obese	54	M
TOX021	211	162.5	36.2	obese	71	M

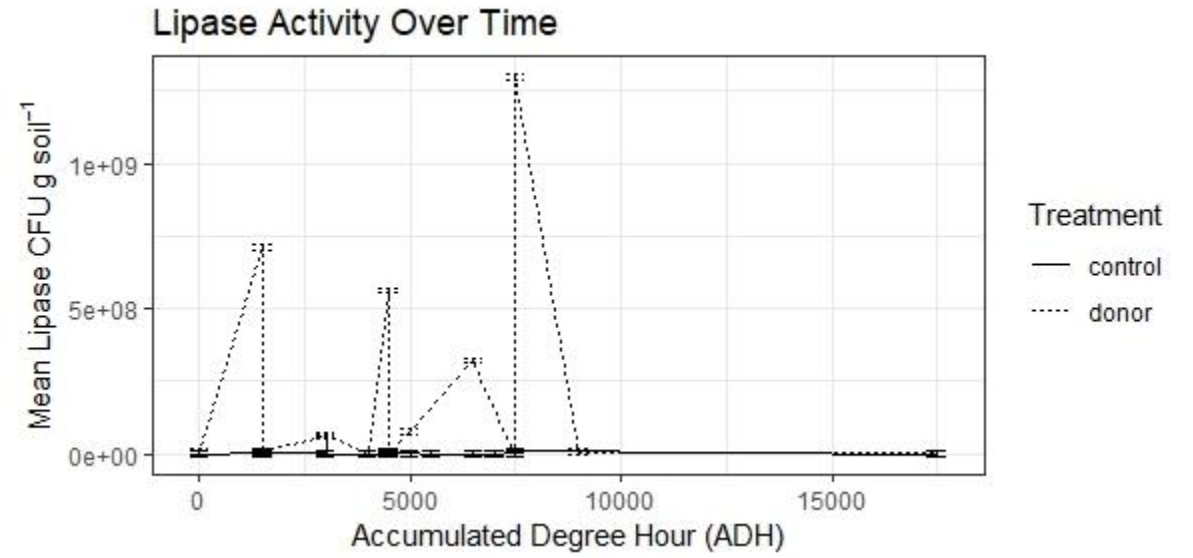

Figure 2. The mean colony forming units (CFU) with lipase activity for each treatment group over time (ADH).

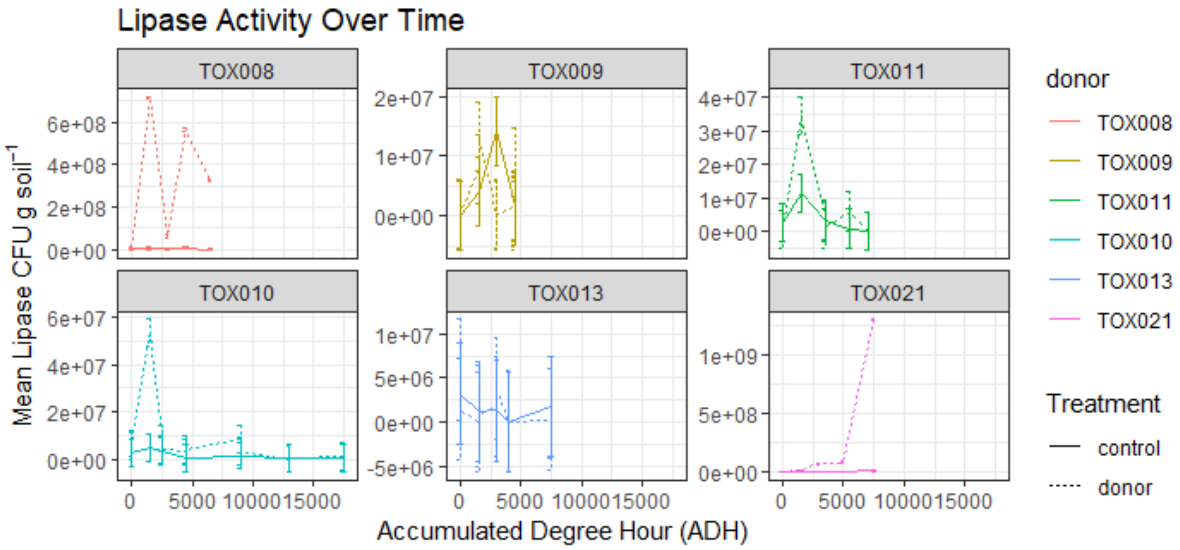

Figure 3. The mean colony forming units (CFU) with lipase activity for each sample over time (ADH). P-values for the difference between control and donor treatments are listed for each donor (TOX008, TOX009, etc.) in Table 2.

Table 2. Two-sample t-tests were performed on the mean CFUs between decomposition soil and the control for each donor, as well as for all donors combined. P-values were the result of t-tests comparing control and decomposition samples for each donor, where an asterisk indicates significance at an alpha value of 0.05.

Donor	**P-Value**
TOX008	0.006*
TOX009	0.213
TOX010	0.006*
TOX011	0.106

TOX013	0.984
TOX021	0.056
All Donors	<0.001*

Results indicated that the relative abundance of lipase CFU was significantly higher under TOX008 ($p < 0.001$), TOX013 ($p = 0.029$), and TOX021 ($p = 0.002$; Table 3) compared to control soils. Overall, the relative abundance of lipase CFU was significantly higher ($p = 0.004$) under donors (mean = 27.71) compared to controls (mean = 22.68) (Table 3), and colonies exhibiting lipase activity was highest between ADH 0 and 3000 for all donors, after which, lipase producing colonies declined (Figure 4).

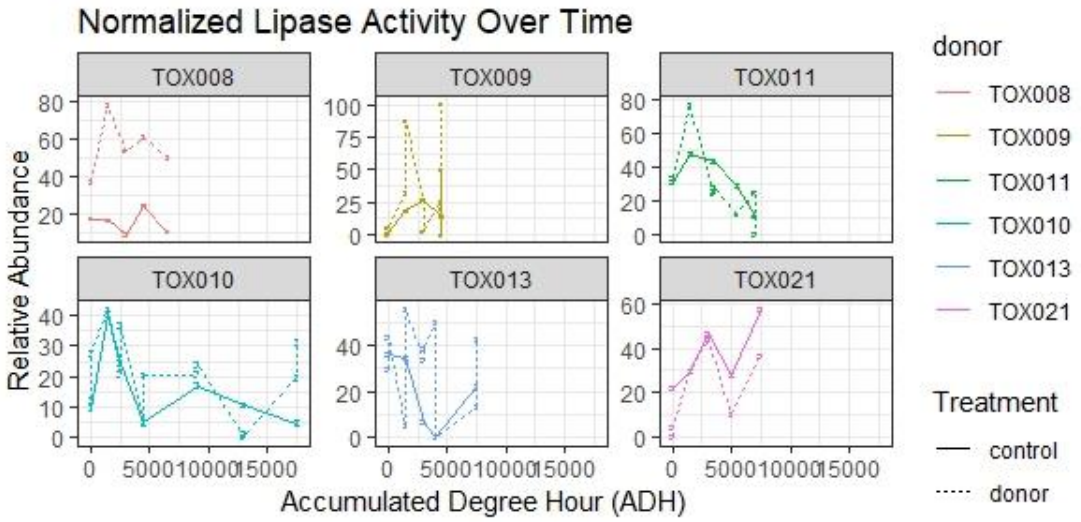

Figure 4. The mean relative abundance of lipase positive colonies for each sample over time. P-values for the difference between control and donor treatments are listed for each donor (TOX008, TOX009, etc.) in table 3.

Table 3. Two-sample t-tests were performed on the relative abundance of lipase CFUs between decomposition soil and the control for each donor, as well as for all donors combined, where an asterisk indicates statistical significance.

Donor	**P-Value**
TOX008	<0.001*
TOX009	0.069
TOX010	0.588
TOX011	0.912
TOX013	0.029*
TOX021	0.002*
All Donors	0.004*

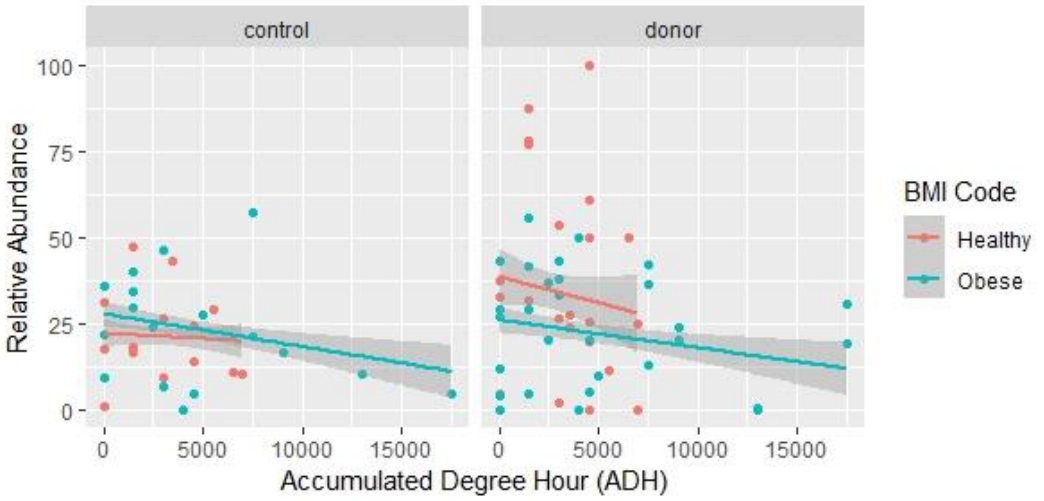

Figure 5. The mean relative abundance of lipase colonies in control and donor soils within healthy (red) and obese (blue) BMI groups over time (ADH).

The relative abundance of lipase CFU was generally higher in healthy donor soils than control soils, whereas obese individuals showed very little deviation between treatment groups (Figure 5). When assessing the relative abundance of lipase colonies beneath either healthy or obese individuals, both control and decomposition soil treatment groups decline over time (Figure 6). Furthermore, the quantity of lipase producing colonies from obese donor soils were more similar to control samples compared to healthy individuals, in which the treatment groups are more easily distinguishable (Figure 6).

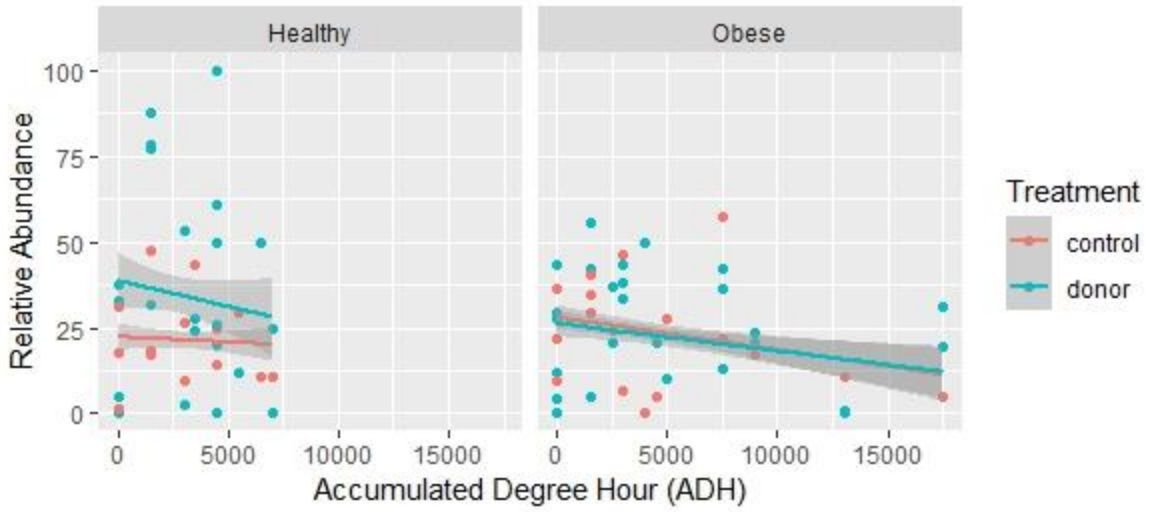

Figure 6. The mean relative abundance of lipase colonies beneath healthy and obese individuals for control (red) and donor (blue) treatment groups over time (ADH).

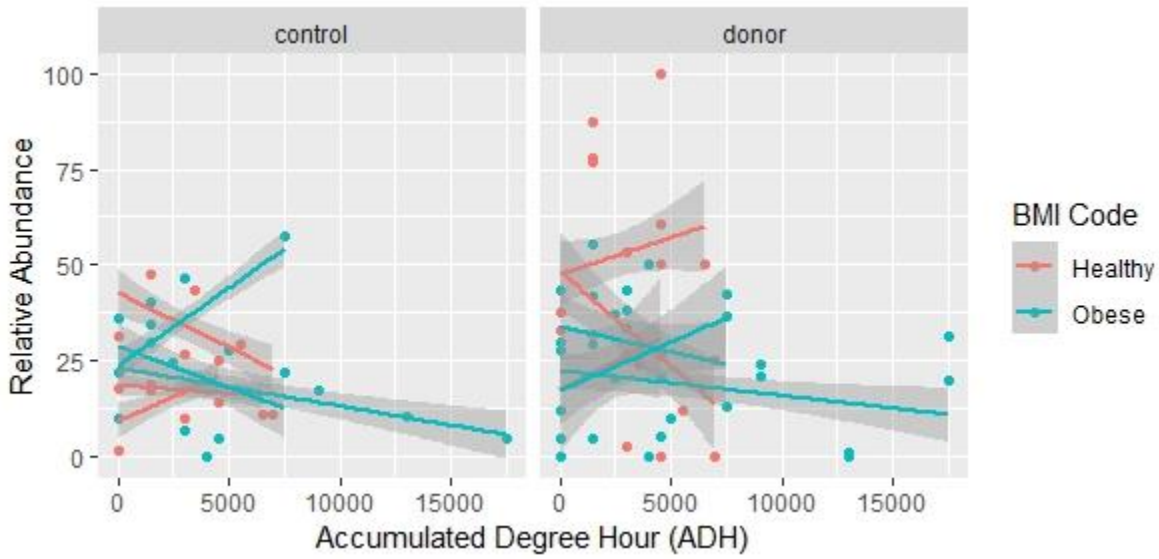

Figure 7. The mean relative abundance of lipase colonies in control and decomposition soils within healthy (red) and obese (blue) BMI groups over time (ADH). Each line represents an individual donor in the respective treatment groups.

Individual donors exhibited unique relative abundance trends, regardless of BMI code (Figure 7). The relative abundance of lipase colonies is higher and more variable in donor soils when compared to controls (Figure 7). In the hierarchical linear mixed-effects model, variation in normalized CFUs was not significantly explained by the variables ADH, BMI code, or the interaction between the two (Table 4).

Table 4: Analysis of variance results from a hierarchical linear mixed-effects model for the response ratio of ADH and BMI code.

Factor	**Sum sq**	**Mean sq.**	**Num DF**	**Den DF**	**F value**	**Pr(>F)**
ADH	0.008946	0.008946	1	3.8976	0.0119	0.9185
BMI Code	0.106912	0.106912	1	4.0416	0.7251	0.7251
ADH : BMI Code	0.093109	0.093109	1	3.8976	0.1238	0.7432

Discussion

The aim of this study was to isolate lipid degrading microbes in human decomposition soils and determine if BMI affects metabolic activity in the soil. Quantity of lipase colonies generally peaked between 0 and 3000 ADH, followed by steady decline. I predicted that soils found below donors with an obese BMI would contain bacterial communities with increased levels of enzymes for metabolizing lipids compared to donors with a healthy BMI. The results indicated that the presence of a decomposing individual (i.e., donor) increased lipase colonies cultured from the soil. These results align with Howard et al. (2010) who found that donors' lipid

content provides additional nutrients to the soil community, and lipase-producing organisms take advantage of this nutrient input. TOX010 had significantly higher bacterial colonies with clearing zones compared to the control and had the highest BMI of the donors sampled. However, TOX008 also had a significantly higher number of colonies with clearing zones compared to the control and had a healthy BMI.

Colony forming units beneath donor soil samples significantly increased compared to controls, regardless of normalization. Prior to normalization of lipase CFU, both TOX008 and TOX010 showed significantly more lipase producing colonies in donor soil samples compared to controls. After normalization, TOX008, TOX013, and TOX021 had significantly more lipase producing organisms in donor soil samples compared to controls. Therefore, TOX008 was the only donor to exhibit significantly increased lipase colonies before and after normalization. Normalization had no effect on trends involving ADH. Overall, microorganisms demonstrating lipase activity was statistically significant in decomposition soils compared to controls, regardless of normalization. However, future studies should still normalize data to account for natural variation between samples.

Healthy individuals had a higher, more distinct relative abundance in decomposition soils, while microbes beneath obese individuals demonstrated lipase activity that was similar to their respective control soils (Figure 5). This finding rejects the initial hypothesis, donors classified as obese based on BMI did not have significantly higher numbers of lipase colonies. This indicates that BMI alone does not explain the increase in lipid degrading microorganisms in the soil below decomposing human carcasses.

Previous research has found that donor BMI does not affect bacterial richness in the soil (Mason et al. 2022), which suggests that BMI does not directly affect the microorganisms in the

soil but simply supplies excess substrate, which is likely the case in this study. Nevertheless, the finding that healthy donors are associated with higher relative abundances of lipase colonies is interesting considering that Mason et al. (2022), studying the same donors, found lower bacterial diversity below healthy donors, whereas diversity remained constant beneath obese donors. The findings presented here may explain the lower diversity under healthy donors because of a higher concentration of lipase producing bacteria; however, further investigation is required.

Individual donors demonstrated unique lipase colony trends both before and after normalization. This study shows that the incipient relative abundance of lipase colonies range between approximately 10% - 45%, regardless of treatment group (control vs. donor soil). Nevertheless, it was found that ADH and BMI code did not significantly affect lipase activity of microbes in soil.

Given that soils beneath obese BMI donors did not contain a significantly larger quantity of lipid degrading microbes despite having an abundance of lipid substrate, other environmental factors may be limiting lipase activity. One possible explanation is the summer sampling season, which has been shown to exhibit lower soil pH below decomposing donors compared to winter months (DeBruyn et al. 2021). Previous research has discovered that the reactivity of lipase tends to decrease as pH decreases because the enzyme enters a denaturation state (Wu 2004). Additionally, aspects of soil physiochemistry, such as nutrient concentrations are altered as a result of summer sampling (DeBruyn et al. 2021). During the summer, decomposition sites experience warmer temperatures, reduced scavenging, and heightened insect activity, all contributing to a more rapid decomposition process (Dautartas et al. 2018). Therefore, extending the decomposition sampling into winter months could provide interesting results in comparison.

One limitation of this study was the small sample size, which may have affected our ability to draw conclusions about the effect of BMI on microbial lipase activity in the soil. Additionally, this study was framed on the assumption that BMI is an accurate measure of body fat, and thus, lipid content. BMI is not a perfect proxy for lipid content, there are cases where high BMI is due to excessive muscle mass and very little lipid content (Meeuwsen et al. 2010). Additionally, studying other BMI categories that are more extreme such as an underweight category could potentially show more definitive results.

Conclusion

The goal of this study was to isolate and quantify lipid degrading microbes in soils beneath decomposing human donors. The aim was to understand how lipase activity in decomposition soils is affected by donor BMI. Results suggest that lipolytic microorganisms are common within soil environments, and the presence of a donor alters the microbial activity and increases the source of lipids to degrade. The presence of a donor was the most significant factor affecting lipase activity in soil ecosystems. However, the finding that donors with a healthy BMI yielded more lipase colonies requires further investigation and may indicate a disconnect between donor BMI and microbial lipase trends. It is also possible that other environmental factors like pH and season are affecting lipase activity in the soil below decomposing human donors.

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References

Dautartas, A., Kenyhercz, M.W., Vidoli, G.M., Meadows Jantz, L., Mundorff, A. and Steadman, D.W., 2018. Differential decomposition among pig, rabbit, and human remains. *Journal of forensic sciences*, *63*(6), pp.1673-1683.

DeBruyn, J.M., Hoeland, K.M., Taylor, L.S., Stevens, J.D., Moats, M.A., Bandopadhyay, S., Dearth, S.P., Castro, H.F., Hewitt, K.K., Campagna, S.R. and Dautartas, A.M., 2021. Comparative decomposition of humans and pigs: soil biogeochemistry, microbial activity and metabolomic profiles. *Frontiers in microbiology*, *11*, p.608856.

Howard, G.T., Duos, B. and Watson-Horzelski, E.J., 2010. Characterization of the soil microbial community associated with the decomposition of a swine carcass. *International Biodeterioration & Biodegradation*, *64*(4), pp.300-304.

Lee, J., Kim, H.S., Jo, H.Y. and Kwon, M.J., 2021. Revisiting soil bacterial counting methods: Optimal soil storage and pretreatment methods and comparison of culture-dependent and-independent methods. *PloS one*, *16*(2), p.e0246142.

Mason, A.R., McKee-Zech, H.S., Hoeland, K.M., Davis, M.C., Campagna, S.R., Steadman, D.W. and DeBruyn, J.M., 2022. Body Mass Index (BMI) Impacts Soil Chemical and Microbial Response to Human Decomposition. *Msphere*, pp.e00325-22.

Meeuwsen, S., Horgan, G.W. and Elia, M., 2010. The relationship between BMI and percent body fat, measured by bioelectrical impedance, in a large adult sample is curvilinear and influenced by age and sex. *Clinical nutrition*, *29*(5), pp.560-566.

Rai, B., Shrestha, A., Sharma, S. and Joshi, J., 2014. Screening, optimization and process scale up for pilot scale production of lipase by Aspergillus niger. *Biomed. Biotechnol, 2*, pp.54-59.

Ramnath, L., Sithole, B. and Govinden, R., 2017. Identification of lipolytic enzymes isolated from bacteria indigenous to Eucalyptus wood species for application in the pulping industry. *Biotechnology Reports*, *15*, pp.114-124.

Wu, H.S. and Tsai, M.J., 2004. Kinetics of tributyrin hydrolysis by lipase. *Enzyme and microbial technology*, *35*(6-7), pp.488-493.