HIGH SCHOOL EDITION
Journal of Student Research
Volume 13 Issue 3 (2024)

Exploring the Co-Occurrence of Methane Oxidation and Bioplastic Genes in Microorganisms

Phillip Jong[1] and Yong-Ju Reichenberger[#]

[1]Ardsley High School, USA
[#]Advisor

ABSTRACT

Methane, a potent greenhouse gas, significantly contributes to climate change. This study investigates the potential of utilizing microbial pathways for converting methane into bioplastics, focusing on the co-occurrence of methane monooxygenase alpha subunit (*mmoA*) and PHA synthase subunit C (*phaC*) genes in diverse microbial strains. We identified and analyzed 43 *mmoA* and 20 *phaC* protein sequences across various phyla, constructing phylogenetic trees and pairwise identity heatmaps to illustrate gene similarities and evolutionary relationships. The results highlight a broad diversity of microbial candidates for methanotrophic bioplastic production, spanning deep-branching groups like *Euryarchaeota* and more evolved taxa such as *Proteobacteria*. Notably, some strains, like *Haloglomus* in *Euryarchaeota*, show promise for bioplastic production under specific environmental conditions, such as high-salt environments. The findings suggest that expanding the range of microbial platforms beyond traditionally studied genera like *Methylosinus* could enhance bioplastic yield and functionality, offering more sustainable and versatile production methods. This study provides a foundation for developing innovative biotechnological solutions to mitigate methane emissions and produce biodegradable plastics, contributing to environmental sustainability and a circular economy.

Introduction

Climate change is a pressing global issue, characterized by its destructive impact on the biosphere and an accelerating rate of change. A significant driver of climate change is the excessive production of greenhouse gases such as carbon dioxide, methane, and nitrous oxide. Greenhouse gases intensify the natural greenhouse effect, contributing significantly to global warming. In 2022, emissions reached an alarming level of 53.85 billion CO_2-equivalent tonnes (Ritchie et al. 2024). Methane stands out as particularly impactful; it is 80 times more potent than carbon dioxide at warming the atmosphere over a 20-year period. Additionally, methane plays a crucial role in the formation of ground-level ozone, further exacerbating climate-related damage (United Nations Environment Programme, 2024).

However, methane can be mitigated through biological methane oxidation (Semrau. 2011, Davamani et al. 2020), or it can be harnessed as a carbon source for synthesizing biodegradable products such as biopolymers or bioplastics (Liu et al. 2020, Gęsicka et al. 2021). Recent advancements indicate that exploiting microbial pathways to convert methane into bioplastics offers significant dual benefits: mitigating methane-induced greenhouse effects and reducing dependency on fossil fuel-derived plastics (Rumah et al. 2021). Additionally, recent research underscores the potential for genetically optimized bacteria to improve both the yield and quality of bioplastics, thereby providing a sustainable alternative to conventional plastic production techniques (Liu et al. 2020, Gęsicka et al. 2021). Such innovations are crucial not only for reducing greenhouse gas emissions but also for advancing toward a circular economy that prioritizes renewable and sustainable materials.

Particularly, two genes are included in this study to explore potential methanotrophic bioplastic production platforms: methane monooxygenase alpha subunit (*mmoA*) and PHA synthase subunit C (*phaC*). These genes are critical for the microbial conversion of methane into biodegradable plastics. Specifically, methane monooxygenase plays a pivotal role in methane oxidation (Sakai et al. 2023), while PHA synthase is essential for polyhydroxyalkanoate (PHA) biopolymer production (Check et al. 2017), which forms the basis of bioplastics.

Currently, a limited number of microbial platforms are being developed for bioplastic production, with species predominantly confined to a few methanotroph genera such as *Methylosinus*, *Methylocystis*, and *Methylobacterium* (Liu et al. 2020, Gęsicka et al. 2021). Considering the vast diversity of untapped microbial groups capable of methane utilization, investigating these candidates could yield more flexible and promising bioplastic production platforms. Furthermore, expanding the search to include a wider variety of microbial groups for methanotrophic bioplastic production will be able to facilitate the creation of high-performing bioplastics. As illustrated in Figure 1, this advancement could pave the way for genetic engineering efforts, leveraging newly identified microorganisms to enhance bioplastic production capabilities.

This research aims to examine the genetic coordination between *mmoA* and *phaC* in various microbial strains to harness their potential for effective methane-to-bioplastic conversion. Based on preliminary information, the following hypotheses were formulated: the common genes that co-occur between methane monooxygenase and PHA synthase will serve as the most proficient candidates for the microbial conversion of methane into biodegradable plastics. It is predicted that, beyond the few known genera, more microorganisms can be explored within other families, orders, and phyla, offering a broader range of candidates for methane-utilizing bioplastic production.

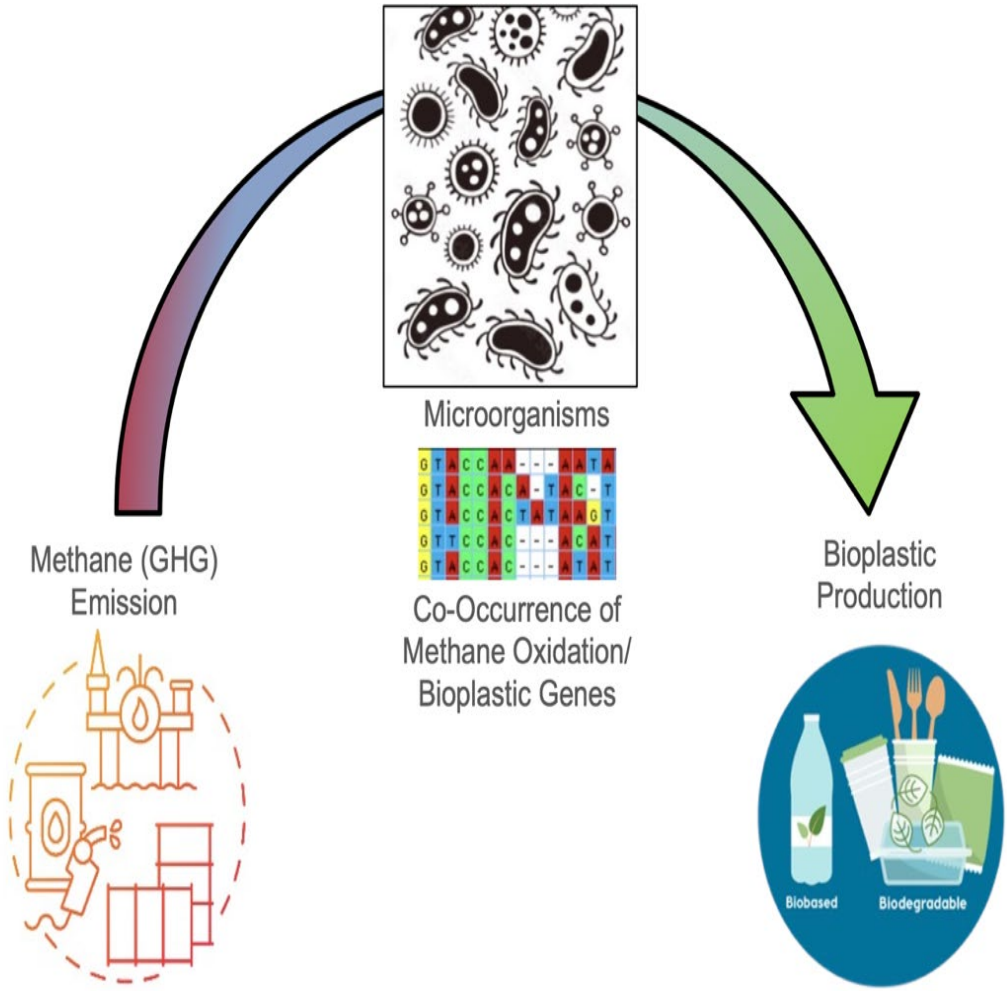

Figure 1. Conceptual illustration of methane-to-bioplastic conversion utilizing microorganisms that possess both methane oxidation and bioplastic production genes.

Methods and Materials

Protein sequences of methane monooxygenase alpha subunit (*mmoA*) were collected from the National Center for Biotechnology Information (NCBI) using the BLAST (GenBank database) tool. The search was performed across all bacterial and archaeal phyla, resulting in 43 sequences from different genera. The *mmoA* gene sequences, ranging from approximately 500 to 600 amino acids, were aligned and analyzed using Molecular Evolutionary Genetics Analysis (MEGA) software (Stecher et al., 2020; Tamura et al., 2021). A phylogenetic tree was constructed employing the Neighbor-Joining algorithm and Poisson correction method.

Similarly, PHA synthase subunit C (*phaC*) genes were collected from the NCBI GenBank database, encompassing 20 different genera. The *phaC* gene sequences, with lengths ranging from approximately 600 to 700 amino acids, were analyzed. The previously collected *mmoA* genes from 43 microbial groups served as queries to verify co-occurrence between *mmoA* and *phaC* genes. Phylogenetic trees for the *phaC* genes were constructed using the same methodology as for the *mmoA* genes.

To assess sequence similarities, pairwise heatmaps were generated using Python 3. The aligned and trimmed sequences of 43 *mmoA* and 20 *phaC* genes were processed with libraries such as Biopython, NumPy,

Seaborn, Matplotlib, Pandas, and SciPy. These libraries and modules facilitated the calculation of sequence similarities and the creation of color-coded heatmaps.

Results and Discussion

Figure 2 presents phylogenetic trees for methane monooxygenase (*mmoA*) and PHA synthase (*phaC*). Among the 43 genera represented in the *mmoA* tree, 20 genera were also found to possess the *phaC* gene, indicating a co-occurrence of these two genes within these microbial groups. The colored lines connecting the identical microbes in both trees highlight the presence of *mmoA* and *phaC* genes in the same genera.

Deep-branching microbial groups such as *Euryarchaeota* (*Haloglomus halophilum*, *Natrarchaeobaculum aegyptiacum*, *Natronolimnohabitans sp.*) and *Acidobacteria* (*Terriglobia sp.*, *Vicinamibacterales bacterium*, *Thermoanaerobaculia bacterium*) are located near the base of both phylogenetic trees. In contrast, more evolved taxa, including *Proteobacteria* (*Methylosinus trichosporium*, *Skermanella stibiiresistens*, *Inquilinus limosus*, *Arboricoccus pini*, *Verminephrobacter eiseniae*, *Pandoraea terrae*, *Burkholderia sp.*, *Paraburkholderia sp.*, *Caballeronia sp.*, *Bryobacteraceae bacterium*) and *Verrucomicrobiota* (*Udaeobacter sp.*, *Chthoniobacterales bacterium*), occupy higher positions in both trees. Additionally, *Bacilliota* (*Neobacillus niacini*, *Paenisporosarcina antarcticais*) are positioned in the middle of these two groups. This distribution illustrates the evolutionary ancestry of *mmoA a*nd *phaC* genes, suggesting that their co-occurrence in the genomes of these microbial groups may have resulted from co-evolutionary processes. The evolutionary pattern observed in these trees supports the hypothesis that the presence of these genes within the same microbial genomes is a product of their shared evolutionary history.

As illustrated in the two tree, multiple microorganisms are involved in methane oxidation or biomolecule production (Sahoo et al. 2021, Safaeian et al., 2023). However, current microbial technology for methanotrophic bioplastic production focuses on only a few taxa, such as *Methylosinus*, as highlighted most frequently in the previous studies (Liu et al., 2020, Gęsicka et al., 2021). Therefore, the newly identified microbial groups for both methane oxidation and bioplastic production in this study suggest a broader diversity, encompassing both deep-branching and more evolved phylogenies. This diversity indicates potential utilization of these candidates in various culture environments. For example, *Haloglomus* in *Euryarchaeota* has been studied as a halophilic organism that thrives in high-salt conditions. To date, as we identified, no studies have demonstrated the use of methane as a feedstock for halophiles, although some halophilic microorganisms are being utilized for bioplastic production (Mitra et al., 2020). Therefore, utilizing methane for bioplastic production with the *Haloglomus* strain identified in this study could provide a novel approach to greenhouse gas mitigation by using methane, in addition to producing biodegradable plastics.

The pairwise identity index shown in Figure 3 provides a direct sequence comparison between the 43 *mmoA* and 20 *phaC* genes. In the heatmap of the *mmoA* gene, more clustering is observed compared to the phylogenetic tree, indicated by the pink and purple colors. The cluster between *Burkholderia* and *Methylosinus* form a large cluster with three internal clusters: (i) *Burkholderia* to *Pandoraea*, (ii) *Inquilinus* to *Methylococcus*, and (iii) *Methylomonas* to *Methylosinus*. This may indicate that the three clusters share similar functions related to methane oxidation within each group. As *Methylosinus* has been most frequently utilized in microbial biomolecule production, similar methane oxidation functions would be expected in *Methylocystis* and *Methylomonas*, which are in the same cluster with *Methylosinus*. Likewise, methane oxidation kinetics would share similar features in the other two mini clusters: (i) *Burkholderia* to *Pandoraea* and (ii) *Inquilinus* to *Methylococcus*, which can be considered for methanotrophic biomolecule production.

In the top left corner of the *mmoA* heatmap, another large cluster of microorganisms is observed, ranging from *Sulfobacillus* at the top to *Sphingobacterium* around the middle. This cluster exhibits high similarities among its members, with colors indicating similarity levels from approximately 60% to 100% (purple, pink, and dark yellow). Despite the diverse microbial groups within this cluster, which include four

different phyla—*Proteobacteria, Acidobacteria, Bacilliota,* and *Planctomycetoa*—there are significant similarities in the *mmoA* gene. This suggests that these diverse taxa may share similar functions in methane oxidation.

The identity index shown in the *phaC* heatmap differs from that in the *mmoA* heatmap. Overall, the similarity index predominantly shows green colors, indicating low sequence similarities between the sequences. Specifically, the heatmap is divided into two large clusters: (i) *Haloglomus* to *Thermoanaerobaculia* and (ii) *Neobacillus* to *Paenisporosarcina*. Notably, the comparison between these two groups shows very low sequence similarities, as indicated by the two green 5×15 rectangular blocks in the top right and bottom left corners. This suggests distinctive functions between the two clusters of PHA synthase. The PHA synthase from *Haloglomus* included in this study was also identified for bioplastic production in similar halophile, *Haloarcula marismortui,* within the same phylum, *Euryarchaeota* (Han et al., 2010). Considering that the PHA synthase from *Haloglomus* belongs to the larger cluster from *Haloglomus* to *Thermoanaerobaculia* in the *phaC* heatmap, we can reasonably assume that the PHA synthases in this cluster have similar functions to those of *Haloglomus*, such as fermentative bioplastic production exemplified by *Haloarcula marismortui* (Han et al., 2010). The smaller cluster from *Neobacillus* to *Paenisporosarcina* contains *Methylosinus*, which has been predominantly used for methanotrophic biomolecule production (Liu et al., 2020; Gęsicka et al., 2021). Similarly, as with the larger cluster, we can expect other strains in the same cluster, such as *Neobacillus*, *Burkholderia*, *Pandoraea*, and *Paenisporosarcina*, to have promising bioplastic production capabilities, such as synthesizing poly-β-hydroxybutyrate-co-hydroxyvalerate (PHBV), as demonstrated by *Methylosinus* (Liu et al., 2020).

Figure 2. Phylogenetic trees of methane monooxygenase alpha subunit (*mmoA*) and PHA synthase subunit C (*phaC*). Different colored lines represent identical microbial taxa that possess both *mmoA* and *phaC* genes. The color code for the corresponding phyla is provided.

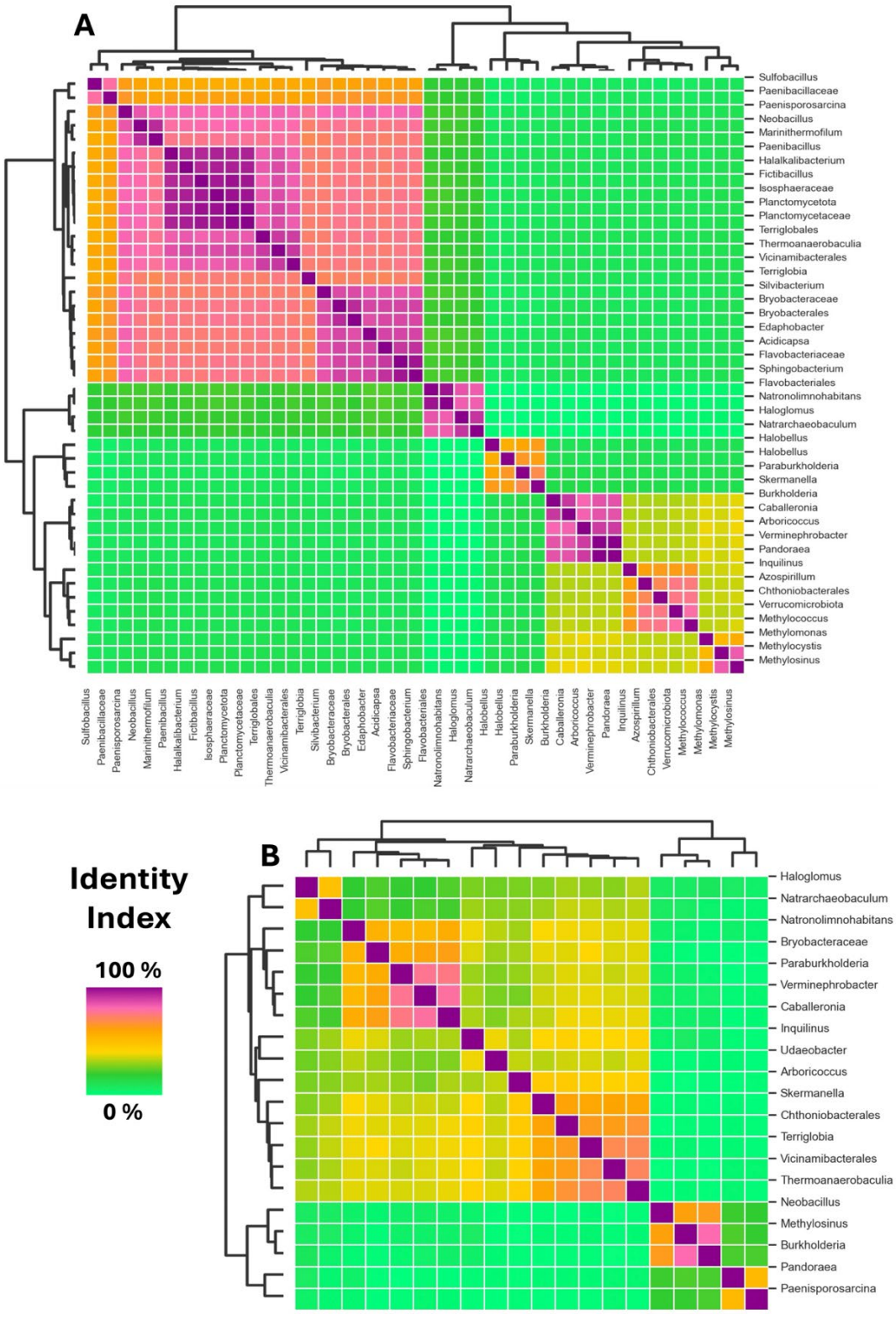

Figure 3. Pairwise identity index heatmaps of methane monooxygenase alpha subunit (*mmoA*) (A) and PHA synthase subunit C (*phaC*) (B). The relative identity index, ranging from 0 to 100%, is represented by various continuous colors for each sequence comparison, with light green indicating 0% and purple indicating 100%.

Table 1. Microbial groups identified with co-occurrence of methane monooxygenase and PHA synthase genes.

Phylum	**Microbial Group**	**Gene Accession Number in NCBI GenBank**	
		Methane Oxidation	**PHA Synthase**
Proteobacteria	*Methylosinus trichosporium*	AAZ81968.1	WP 338065165.1
	Skermanella stibiiresistens	WP 201078147.1	WP 084164931.1
	Inquilinus limosus	WP 088149201.1	WP 034846533.1
	Arboricoccus pini	WP 088561902.1	WP 088560549.1
	Verminephrobacter eiseniae	WP 011812099.1	WP 265304547.1
	Pandoraea terrae	WP 150698856.1	VVE56757.1
	Burkholderia sp.	EEA01877.1	WP 124601950.1
	Paraburkholderia sp.	WP 321939055.1	HWT38593.1
	Caballeronia sp.	WP 250520497.1	WP 299713149.1
	Bryobacteraceae bacterium	MDR3719414.1	HXJ43377.1
Acidobacteria	*Terriglobia sp.*	HTG 61963.10	MCL6490298.1
	Vicinamibacterales bacterium	HWW84847.1	HYJ94124.1
	Thermoanaerobaculia bacterium	HKO57199.1	KAB2969312.1
Bacillota	*Neobacillus niacini*	WP 335417253.1	MDR6999156.1
	Paenisporosarcina antarctica	WP 243840709.1	WP 134209868.1
Euryarchaeota	*Haloglomus halophilum*	WP 254822921.1	WP 254824368.1
	Natrarchaeobaculum aegyptiacum	WP 086889112.1	WP 086886896.1
	Natronolimnohabitans sp.	WP 306759918.1	WP 306758986.1
Verrucomicrobiota	*Udaeobacter sp.*	HXM26056.1*	HXQ42610.1
	Chthoniobacterales bacterium	HXM26056.1*	HYY30115.1

Table 1 summarizes the candidates that can be utilized for methanotrophic bioplastic production. These microbial groups are dispersed across various phyla and possess both methane oxidation (*mmoA*) and PHA synthase (*phaC*) genes. This indicates their potential as platforms for methanotrophic bioplastic production in diverse environments or cultivation conditions. For example, Safaeian et al. (2023) discussed bioreactor configurations using *Methylosinus* to achieve higher growth kinetics and bioplastic yield. These conventional bioreactor designs could be enhanced by utilizing the newly identified microorganisms that possess both *mmoA* and *phaC* genes.

This finding also supports the utilization of more diverse feedstocks with methane (Semrau, 2011). Given the diverse niches of the newly identified microorganisms, their nutrient and mineral requirements could vary. This suggests that more economical options could be pursued when designing methanotrophic bioplastic production platforms.

Future research efforts should include identifying the co-occurrence of the two genes (*mmoA* and *phaC*) at the strain level, as most of the exploration in this study was conducted at the genus or species levels or higher. Additionally, it is essential to confirm not only the presence of *mmoA* and *phaC* but also the entire pathways for methane monooxygenase and PHA synthase in these selected strains. This can ensure robust microorganisms and the platforms they play, ultimately achieving the dual goals of greenhouse gas mitigation and nature-based material production.

Conclusion

This study highlights the potential of leveraging diverse microbial groups for methane-to-bioplastic conversion by examining the co-occurrence of *mmoA* and *phaC* genes. The phylogenetic analysis and pairwise identity heatmaps reveal significant genetic diversity among the candidate microorganisms, suggesting their suitability for various environmental conditions and bioplastic production requirements. Particularly, strains like *Haloglomus* demonstrate promising bioplastic production capabilities in high-salt environments, indicating the feasibility of utilizing a broader range of microbial platforms. The integration of newly identified microorganisms could enhance current bioreactor configurations, improving growth kinetics and bioplastic yield. Additionally, this approach supports the use of diverse feedstocks, potentially reducing production costs and contributing to more sustainable bioplastic manufacturing. Future research should focus on strain-level identification of gene co-occurrence and confirmation of entire pathway analysis for methane monooxygenase and PHA synthase in the selected strains. By advancing our understanding of microbial methane utilization and bioplastic synthesis, this study paves the way for innovative strategies to mitigate greenhouse gas emissions and promote the development of renewable, biodegradable materials.

Acknowledgments

I would like to thank my advisor, Mrs. Reichenberger.

References

Chek, M. F., Kim, S.-Y., Mori, T., Arsad, H., Samian, M. R., Sudesh, K., & Hakoshima, T. (2017). Structure of polyhydroxyalkanoate (PHA) synthase PhaC from Chromobacterium sp. USM2, producing biodegradable plastics. *Scientific Reports, 7*, 5312. https://doi.org/10.1038/s41598-017-05509-4

Davamani, V., Parameswari, E., & Arulmani, S. (2020). Mitigation of methane gas emissions in flooded paddy soil through the utilization of methanotrophs. *Science of the Total Environment, 726*, 138570. https://doi.org/10.1016/j.scitotenv.2020.138570

Gęsicka, A., Oleskowicz-Popiel, P., & Łężyk, M. (2021). Recent trends in methane to bioproduct conversion by methanotrophs. *Biotechnology Advances, 53*, 107861. https://doi.org/10.1016/j.biotechadv.2021.107861

Han, J., Hou, J., Liu, H., Cai, S., Feng, B., Zhou, J., & Xiang, H. (2010). Wide distribution among halophilic archaea of a novel polyhydroxyalkanoate synthase subtype with homology to bacterial type III synthases. *Applied and Environmental Microbiology, 76*(23), 7811-7819. https://doi.org/10.1128/AEM.01117-10

Liu, L.-Y., Xie, G.-J., Xing, D.-F., Liu, B.-F., Ding, J., & Ren, N.-Q. (2020). Biological conversion of methane to polyhydroxyalkanoates: Current advances, challenges, and perspectives. *Environmental Science & Ecotechnology, 2*, 100029. https://doi.org/10.1016/j.ese.2020.100029

Mitra, R., Xu, T., Xiang, H., & Han, J. (2020). Current developments on polyhydroxyalkanoates synthesis by using halophiles as a promising cell factory. *Microbial Cell Factories,19*(86). https://doi.org/10.1186/s12934-020-01342-z

Rumah, B., Stead, C. E., Stevens, B. H. C., Minton, N. P., Grosse-Honebrink, A., & Zhang, Y. (2021). Isolation and characterisation of Methylocystis spp. for poly-3-hydroxybutyrate production using waste methane feedstocks. *AMB Express, 11*, 6. https://doi.org/10.1186/s13568-020-01159-4

Sahoo, K. K., Goswami, G., & Das, D. (2021). Biotransformation of methane and carbon dioxide into high-value products by methanotrophs: Current state of art and future prospects. *Frontiers in Microbiology, 12*, 636486. https://doi.org/10.3389/fmicb.2021.636486

Safaeian, P., Yazdian, F., Khosravi-Darani, K., Rashedi, H., & Lackner, M. (2023). P3HB from CH4 using methanotrophs: Aspects of bioreactor, fermentation process, and modeling for cost-effective biopolymer production. *Frontiers in Bioengineering and Biotechnology, 11*, 1137749. https://doi.org/10.3389/fbioe.2023.1137749

Sakai, Y., Yurimoto, H., & Shima, S. (2023). Methane monooxygenases; physiology, biochemistry and structure. *Catalysis Science & Technology, 13*, 6342. https://doi.org/10.1039/d3cy00737e

Semrau, J. D. (2011). Bioremediation via methanotrophy: Overview of recent findings and suggestions for future research. *Frontiers in Microbiology, 2*, 209. https://doi.org/10.3389/fmicb.2011.00209

Stecher, G., Tamura, K., & Kumar, S. (2020). Molecular Evolutionary Genetics Analysis (MEGA) for macOS. *Molecular Biology and Evolution, 37*(4), 1237-1239. https://doi.org/10.1093/molbev/msz312

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution, 38*(7), 3022-3027. https://doi.org/10.1093/molbev/msab120

United Nations Environment Programme. (2024). Methane emissions are driving climate change. Here's how to reduce them. UNEP. Accessed on May 2024. https://www.unep.org/news-and-stories/story/methane-emissions-are-driving-climate-change-heres-how-reduce-them#:~:text=What's%20the%20big%20deal%20about,also%20a%20powerful%20greenhouse%20gas.

Ritchie, H., Rosado, P., & Roser, M. (2020-2024). Greenhouse gas emissions. Published online at OurWorldInData.org. This page was first published in June 2020 and last revised in January 2024. Accessed on May 2024. https://ourworldindata.org/greenhouse-gas-emissions