Qeios

Peer Review

Review of: "Polyethylene Terephthalate (PET) Primary Degradation Products Affect c-di-GMP-, cAMP-Signaling, and Quorum Sensing (QS) in Vibrio gazogenes DSM 21264"

Sabrina Edwards[1]

1. Reed College, United States

Dear Authors,

Thank you for the opportunity to review your manuscript, *Polyethylene Terephthalate (PET) Primary Degradation Products Affect c-di-GMP-, cAMP-Signaling, and Quorum Sensing (QS) in Vibrio gazogenes DSM 21264.* In an era where mass-produced plastics increasingly enter natural ecosystems, your contributions to understanding plastic biodegradation are highly valuable and insightful. I am pleased to provide additional feedback; below, I provide comments and suggestions that I hope are helpful.

General Comments

Your investigation into biofilm formation and signaling dynamics raises important questions regarding PET degradation in *Vibrio gazogenes* DSM 21264. The study aims to address several key questions:

1. Is *PET6* expressed under environmentally relevant conditions?
2. If so, can *PET6* degrade PET under these conditions?
3. Is *PET6* expression dependent on PET or its byproducts (e.g., BHET, TPA, EG)?

These questions are crucial for understanding the metabolic and regulatory mechanisms driving PET biodegradation. Your study provides new insights into how PET and its degradation byproducts interact with microbial signaling pathways, which has important implications for PET biodegradation research. However, several areas identified below may benefit from more clarification.

Major Comments

doi.org/10.32388/O3CLMY

- **BHET Concentration Selection:**
  - The rationale behind selecting 0.5, 5, and 30 mM BHET concentrations is unclear. Are these physiologically relevant concentrations derived from expected hydrolysis?
  - In *Figure 3*, alternative carbon sources are tested at 1% w/v, while BHET (0.76% w/v at 30 mM) and TPA (0.02% w/v at 1 mM) are much lower. Could substrate concentration affect gene transcription and survival/phage-related gene expression rather than substrate type?
- **MHET as a Potential Signaling Molecule:**
  - Since MHET is the predominant degradation product observed in the biofilm matrix, why was it not further investigated for its role in signaling and regulation? If BHET accumulation is not observed during the degradation process, its transcriptional influence should be clarified.
- **Contradiction in EG Utilization:**
  - The manuscript states that *adhE* is detected in the transcriptome but concludes that ethylene glycol (EG) cannot serve as a sole carbon source. Given *Figure 7*'s proposed metabolic role for *adhE*, this discrepancy should be addressed.
- **Interplay Between PET Degradation and Biofilm Formation:**
  - The manuscript states that DSM 21264 does not use PET byproducts as sole carbon sources. Does this suggest that DSM 21264 degrades PET but does not utilize it for growth?
    - Could this imply interspecies interactions in biofilms, particularly given BHET's effect on quorum sensing (QS) and signaling?
- **Role of UlaG in BHET Metabolism:**
  - The function of *UlaG*, a predicted metallo-β-lactamase, in BHET metabolism requires further clarification. Is its expression merely co-regulated, or does it actively hydrolyze BHET? Is there a control group showing that BHET hydrolysis observed is a result of enzymatic activity rather than acid-induced hydrolysis due to pH changes? Is there a proposed mechanism for how it could degrade BHET?
- **Biofilm vs. Planktonic Transcriptional Differences:**
  - The authors report that µM amounts of TPA and MHET appear in biofilms within 24 hours, suggesting that gene expression may respond to oligomers/monomers in biofilms rather than in planktonic cells.
    - Transcriptional differences between biofilm and planktonic conditions are briefly addressed: *"As expected, planktonic cultures exhibited a largely different transcriptional pattern compared to biofilms (Table S1, Figures S4–6, & Figure S2)."* However, much of this data is in supplemental

materials rather than the main text. A more thorough discussion and rationale within the manuscript would

Conclusion

This study provides valuable insights into biofilm-associated PET degradation, but several areas require further clarification and potentially additional experimental controls. Addressing these points will strengthen the manuscript's conclusions.

Declarations

Potential competing interests: No potential competing interests to declare.