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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"

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Thank you for the opportunity to review this manuscript. The manuscript reports on the proposed mechanism of PET biodegradation in the marine bacterium *Vibrio gazogenes* DSM 21264. The authors investigate how *pet6* (a PET hydrolase enzyme) is expressed in the presence of plastic substrates and analyze transcriptomic data to identify other genes involved in biodegradation. The results provide novel insights into interactions between PET, its degradation byproducts, and microbial signaling pathways. This study is particularly noteworthy as *Vibrio* spp.—rarely studied as plastic degraders—are recognized as primary colonizers of microplastics (PP, PE, PS) in marine plastispheres. These findings have significant implications for PET biodegradation research and shed light on the ecological role of *Vibrio* in plastisphere communities.

The manuscript presents compelling results and a coherent narrative. Below are suggestions to strengthen the work:

Comments/Questions:

1. **Normalization with *E. coli* DH5α:**

   The rationale for using *E. coli* DH5α (a Gram-negative strain lacking PET/PE-degrading genes) as a control instead of an abiotic control is unclear. Clarify this choice to improve transparency. For example:

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"Since no PET/PE-degradative genes have been reported in E. coli *DH5α, this strain was selected as a negative control to account for background metabolic activity."*

2. **Planktonic vs. Biofilm Stage Determination:**

   The methodology for distinguishing planktonic and biofilm stages (e.g., microscopy or staining) is not described. Include this in the *Methods* or *Results* to ensure reproducibility.

3. ***adhE* Gene Expression Analysis:**

   a. The *adhE* gene (upregulated in PET biofilms) is absent from Table S3. Clarify whether this omission reflects a statistical cutoff issue or a methodological oversight.

   b. Justify the use of reporter fusion for *adhE* validation. Is this due to low confidence in transcriptomic data or an effort to confirm transcriptional activity *in situ*?

4. **BHET Concentration Rationale:**

   The choice of BHET concentrations (0.5, 5.0, 30 mM) needs clarification. Are these levels ecologically relevant to plastispheres, or are they based on prior studies of DSM 21264? For example, in Figure S4, 5 mM BHET degradation was observed and studied; were lower and higher concentrations selected to explore the strain's biodegradation capacity beyond this midpoint?

5. **Figure/Table Reorganization to Streamline Key Findings:**

   The lack of graphical figures in the main text may hinder reader comprehension. To address this, I suggest:

   - Move **Figure S3** to the main text (demonstrating UlaG and PET6 as BHET hydrolases in DSM 21264).
   - Move **Figure S4** to the main text (demonstrating 5 mM BHET biodegradation by DSM 21264).
   - Move **Figure S5** to the main text (demonstrating effects of various BHET and TPA concentrations on biofilm formation and colony morphology).
   - Relocate **Table 1** to the supplementary materials, as the corresponding results are already shown in the supplement.
   - Group **Figures 4–6** to consolidate signaling pathways (c-di-GMP, cAMP-CRP, QS) affected by BHET/TPA for better clarity.

6. **PET/PE Foil Specifications:**

   Include material details (e.g., thickness, crystallinity, supplier) for PET/PE substrates. This is critical for reproducibility and comparison with other studies.

Declarations

Potential competing interests: No potential competing interests to declare.