

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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1. Independent researcher

Dear Sir/Madam,

The manuscript from Preuss et al. provides sufficient experiments regarding *Vibrio gazogenes* DSM 21264 in both biofilm and planktonic lifestyles. Furthermore, evidence is provided that *pet6* transcription does not discriminate between PET and PE surfaces under biofilm conditions, but it reaches the highest point for the planktonic cells growing in the presence of BHET. For the first time, it is shown that PET6 and the lactonase UlaG are described as potential BHETases, but higher concentrations of BHET affected quorum sensing, c-di-GMP, and cAMP-CRP signaling. Consequently, the biofilm formation, cell morphology, and synthesis of prodigiosin were also affected. Another PET degradation product, TPA, was observed to interfere with quorum sensing. These findings help to increase our current understanding of *Vibrio gazogenes* colonization of the plastisphere.

That being said, I think the text needs some modifications to help the reader understand the main messages. I separated my notes according to each topic. Please note that I did not use artificial intelligence to aid my revision.

Abstract:

L3: please replace "We recently showed that" with "Recently, we showed that".

L4: "the *pet6* gene" could be simply replaced by "*pet6*", because it is in italics and, therefore, it is assumed to be the gene. Please note that this is valid for the whole manuscript.

L6: replace “affected by” with “influenced by”

L8: I find “ μM amounts” very confusing for the reader. Please be more specific about how many μM . I can totally understand if it was not measured; in this case, I would simply remove it and leave “release of MHET and TPA”. Please note that this is valid for the whole text.

L9: Was it PET amorphous foil? If yes, I would add this extra information.

L9: Replace “highest” with “optimal”

L10: replace “was observed in planktonic lifestyle” with “was observed for planktonic cells”

L11: Remove “one of the primary degradation products of PET,” because this can be further mentioned in the introduction or results alongside TPA, for example.

L11-12: Please highlight the results obtained for UlaG by using the phrase “Additionally, BHET was further hydrolyzed by PET6 and UlaG, a lactonase that was not previously reported to degrade BHET.”

L13-16: Merge two phrases together by using “which”: “Elevated concentrations of BHET affected the major signaling circuits involved in bacterial quorum sensing (QS), c-di-GMP and cAMP-CRP signaling, which resulted in a failure to form biofilms, as well as the synthesis of the red pigment prodigiosin and altered colony morphologies.”

L17: Replace “While BHET had a very wide impact” with “On the other hand,”

L20: replace “and that may affect its growth and survival in the plastisphere.” with “, which might affect its growth and survival in the plastisphere.”

Importance:

L2: replace “and degrades the polymer using PET6 when growing in biofilms on foils and microplastic particles.” with “, capable of degrading this polymer when the biofilm grows on microplastic particles.”

L5: replace “may” with “might”

Introduction:

L1: replace “in the recent decade” with “in the last decades”

L4: add “known as macroplastics” after “larger floating fragments”

L5: replace “with sizes” with “measuring”

L6: Modify the phrase to: “These particles contain additives that could have a negative impact on all ecological niches and their biodiversity.”

L7: replace “they will” with “micro-, nano-, and pico-plastics were also reported to affect human health and wellbeing.”

L10: write “polyvinyl chloride” separately. Maybe put the polymers in the order from highest to lowest in production.

L11: what do you mean by “only being produced at lower quantities”?

L12-15: rewrite to “However, the enzymes and their respective native hosts capable of degrading these synthetic polymers are not well-known, apart from PET (www.PAZy.eu).”

L16: delete “all”, leaving “that have a wide substrate specificity”

L17: delete “recently”, leaving “much effort has been made”

L18: replace “phylogenetic makeup” by “phylogeny”

L17-20: These two phrases separated by a point could actually be merged, as they provide the same information: “In addition to these studies, much effort has been made to analyze and characterize the phylogeny of microbial communities associated with plastic particles found in the marine environment. The microorganisms colonizing plastic particles (i.e. the plastic microbiota) have been termed ‘plastisphere’.”

L21-25: delete “growth on and” as the growth is already implied. Here, the two phrases separated by a point also present the same idea and could also be merged.

L23: “of the various polymers”: which ones?

L24: please change to “distinct chemical composition of additives play a key role”

L27-28: delete “observed” and “in their phylogeny”, because it is already implied. Leave “The microbial communities are highly diverse and bacteria affiliated with the genus *Vibrio* are often observed on plastic particles”

L29-30: write “*Vibrio* species are ubiquitous gram-negative bacteria, but mainly found in marine environments where they are considered to be key players in carbon and nitrogen cycling.” The addition of “but” as a connector gives the idea that, although they are ubiquitous, they still have preferences.

L30-32: add “Currently,” and “are described to be” as in the final phrase proposal: “Currently, there are more than 100 known *Vibrio* species of which few are described to be either human or fish pathogens (e.g. *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Vibrio vulnificus* and others)”

L33: “recently, we showed that” instead of “we have shown”

L34-35: “has a genome size of 4.6 Mbp and harbors a gene encoding a promiscuous esterase designated PET6” instead of adding “in its 4.6 Mbp genome” at the end of the phrase.

L35: what do you mean by “(synonym PB1, AATCC 29988)”?

L36: delete “gram-negative”, as you already mentioned above. Leave “is a non-pathogenic bacterium, which was isolated from”

L37-38: Change the following sentences to “Furthermore, this species is typically found in estuaries and produces a red pigment named prodigiosin”

L39: “the gene *pet6*” instead of “the *pet6* gene”

L40-43: Modify to “The enzymatic activity is salt-dependent, and the hydrolysis of PET is rather low when compared to other known enzymes used in industrial processes, for instance, the well-characterized IsPETase or LCC.”

L43: Add the references of IsPETase and LCC

L43: replace “While much information was gathered on the enzyme structure and biochemical function of PET6,” with “Despite the information regarding the structure and biochemical function of PET6,”

L45-47: change to “it was not known under which conditions *pet6* could be transcribed in its native host, and if the wild type enzyme could degrade PET in the environment.”

L49-51: Use however at the beginning of the phrase and make a few modifications: “However, the gene expression is neither affected by the presence of PET and PE foils nor PET powder.”

L51: replace “instead” with “alternatively”

L52: delete “further”, leaving “our data imply...”

L54-55: The last phrase of the introduction needs appropriate references.

Results:

Suggestion of a new title: "DSM 21264 forms patchy biofilms on PE and PET foils without surface preferences and releases MHET and TPA"

L1: Add a comma after "hydrolase"

L2: "this microorganism" instead of "the organism"

L4: suggestion of abbreviation to "ASW" medium, instead of "ASWM". You may want to check which abbreviation appears frequently on PubMed. This is valid for the whole manuscript.

L5: replace "Since no PE-degradative genes have been reported in gram-negative bacteria" with "Because the genes encoding PE-degradation enzymes were not previously reported in gram-negative bacteria,".

L7: Delete the hyphen between "PE foil". Was PE foil considered a negative control?

L7-8: instead of "in these biofilm experiments" use "In general, after 10 and 180 days,"

L8-9: Replace the plural word with the singular "the cells attached at very low frequency and only a thin single cell layer biofilm was formed (Figure 1 & S1)."

L9: replace "therefore" with "For this reason"

L11: add "in order" to "in order to promote growth and biofilm formation."

Suggestion of changes in the legend of Figure 1: "Figure 1. Scanning electron microscopy (SEM) (A, D) and confocal laser scanning microscopy (CLSM) images (B, C, E, F) of *V. gazogenes* DSM 21246 biofilms on PET and PE foil. SEM images and CLSM images (C & D) show the first attachment of cells after 8 hours of incubation in ASW medium at 22 °C on PET foil (A & C) and PE foil (B & D). CLSM images (E & F) show biofilm formation of DSM 21264 on PET foil (E) and PE foil (F) after 24 hours of incubation. Cells were stained using LIVE/DEAD stain."

L13: regarding the phrase "However, the net-like structures were more prominent on PE surfaces (Figure 1).", does it show a preference for this substrate?

L14: What are the applications of air plasma treatment?

L15-18: place a comma after "Additional tests with air plasma-treated PET and PE foil were conducted" and include "because this treatment leads to the formation of oxygen-functionalities and thus an increase in surface polarity, which typically gives a putative degradation enzyme better access to the polymer fibers"

L19: replace “only minor differences were observed with respect to biofilm formation (Figures S 1 & S1).” with “The biofilm formation was slightly different in the experiments performed with PET and PE foil pre-treated with air plasma,”. Also, with this minor difference observed, did they benefit from the biofilm formation or not?

When you cite a supplementary Figure, the following format is shown: “Figures S 1 & S1”. Please fix it accordingly.

L21-23: replace “Notably, when we grew” with “Remarkably, DSM 21264 growing on PET foil or powder released detectable μ M amounts of MHET and TPA in the biofilm matrix and culture supernatants after 24 hours (Figure 2).”

Proposed new legend for Figure 2: “Figure 2. PET degradation products observed in supernatants of DSM 21264 biofilm on PET foil (non-treated), UV-treated foil, and PET powder. DSM 21264 was grown in ASWM with a limited carbon source, and the supernatants were collected after 1, 3, and 5 days. The degradation products were measured using UHPLC, as described in the Material and Methods section. The data were normalized and corrected to *E. coli* DH5 α growing on PET foil and powder. Data are mean values of three independent replications, and bars represent the standard deviations.”

I have a few questions regarding the experiments of Figure 2:

“PET foil (non-treated)” do you mean “non-treated with UV light”?

“UV-treated foil” do you mean “UV-C treated foil”?

UHPLC abbreviation needs to be fully written, also in the text when appearing for the first time.

“The data was normalized and corrected to *E. coli* DH5 α growing on PET foil and powder.” Why were these conditions chosen for the normalization and correction?

L24: replace “if the” with “whether”

L25: replace “or” with “and”, because it sounds more formal. It is already clear that you did not use them simultaneously.

L28: replace “or” with “and”; replace “during life” with “in both biofilm and planktonic lifestyles”

L29-30: connect the phrases using “and”, leaving “and each experiment was repeated three times.”; write “three” instead of “3” and check for similar mistakes among the text.

L30-32: change to “The resulting 42 RNAseq data sets were submitted to the European Nucleotide Archive (ENA) and they are publicly available under the accession number PRJEB80907.”

L32: start the sentence with “Besides, the obtained reads”

L34-35: delete “for” after “encoding for”

L35-39: I suggest small changes in the following sentences: “The newly established genome sequences are available under the accession numbers CP151640 and CP151641, respectively, at NCBI/GenBank. An overview of the data sets obtained, the percentage of reads mapped to the genome and other parameters together with the level of *pet6* expression is given in Table S2 and Figure 3.”. I also suggest that you write in the format GenBank (National Center for Biotechnology Information, NCBI).

Suggestion for Figure 3: replace “all 4,117 genes” with “the 4,117 genes in all of the 42 transcriptomes.”

Suggestion for the subtitle: replace “grown” with “formed”

L40: start with “When comparing PET- *versus* PE-grown biofilms,”. *Versus* is Latin and needs to be written in italics.

L43: replace “level of expression” with “expression level”

L44: replace “all genes” with “every gene”

L44: start the sentence with “Air plasma-treatment of PET and PE foil”

L45: biofilms in plural and delete the word “cultures”

L45: please be more specific with “and as described above”

L46: remove the comma, leaving “In addition, we noticed that in all biofilm experiments the”

L47: replace “possibly is” with “might be”

L51: replace “in addition” with “The gene *adhE*”

L52: replace “but not in PE-grown biofilms” with “only”, because it is a little bit repetitive and hard to follow

L54: “The promoter was fused with the amcyan gene in pBBR1-MCS-1 vector”

L55-57: please rewrite: “With the transcriptional reporter *P_{adhE}::amcyan* in DSM 21264, we were able to verify that *adhE* was 2-fold upregulated in the presence of 5 mM EG (Figure S5).”

Suggestions for Table 1: instead of “strains or plasmids” use “strains/plasmids”

The subtitles “strains” and “plasmids” in italics are not good because they are mixed with the genes. I suggest adding them also in bold.

L58: Usually, instead of once, it is used “since”. Please replace here and in other parts of the document.

L58: delete the word “other” from “we further asked if other natural”, because it is the first time that you start mentioning natural polymers.

L60: replace “assayed the transcriptomes” with “collected for transcriptome analysis”

L61: “as expected for” instead of “as expected in”

L62: add “when” to “was observed when compared to the biofilm conditions”

L63: replace “remarkably” with “remarkable”

L66: What is the function of *rpoD*? I believe it is a housekeeping gene

L67: delete “any of” and “added”. Also, do you mean “comparable” instead of “compared”?

L68-69: In the phrase “However, the expression was significantly higher in the planktonic cultures compared to the biofilm cultures (Figure 3),” maybe add the information “when the natural polymers were added.”

L72: Which liquid media? All of the ones tested in this work?

L73-74: Rephrase to the following: “The overall gene expression profile was analyzed using RNAseq.”

L75-76: “Liquid cultures” is “ASWM”? Also, replace “the presence of” with “liquid cultures supplemented with.”

L76-78: Merge two phrases, as in “Table S2 summarizes the data obtained for all RNAseq experiments, whereas Figure S2 displays volcano plots that highlight major changes in gene expression.”

L81-87: Rewrite for improved clarity: “The higher concentrations (5.0 and 30 mM) of BHET had the most pronounced effects on the overall gene expression levels in DSM 21264, with >230 and >830 upregulated in the presence of 5.0 mM and 30 mM of BHET, respectively. On the other hand, >100 and >550 genes were downregulated in the presence of 5.0 mM and 30 mM of BHET, respectively. These results were obtained with the comparison to the DMSO and the TPA controls, which are displayed in Figure S7.”

L88: Replace “is most likely” with “might be.”

L89-91: Rewrite for better clarity: “This is in line with an almost 5-fold (log₂-foldchange) increased transcription level of *pet6* (AAC977_05355) observed for the culture supplemented with 5.0 mM of BHET and a 1.8-fold increase at 30 mM.”

L91-98: Please rewrite to “Surprisingly, the RNAseq data indicated that the predicted metallo- β -lactamase *UlaG* is also involved in BHET metabolism. The strongest transcription of *ulaG* was observed in the presence of 30 mM BHET, with a 7.9-fold upregulation when compared to the controls. To further verify this novel role of *UlaG*, we expressed the recombinant protein in *E. coli* T7 SHuffle and evaluated the hydrolysis of BHET, which showed that this promiscuous enzyme was able to cleave BHET at slow but significant rates (Figure S3).”

L101: The phrase “It does not encode any mono- and dioxygenases involved in aromatic ring cleavage” needs a reference. It also needs to be linked to the previous phrase.

L103: Replace “affecting” with “that affected.”

L104: Replace “BHET altered colony morphology already at rather” with “The colony morphology was already impacted at a lower concentration (0.5 mM), as the colonies were less structured and had smooth edges.”

L108: Replace “present” with “supplemented.”

L113: Remove the first time that the word “signaling” appears, because it is repetitive.

L113-115: The phrase “These pathways were chosen, as they are known to have a wider impact on biofilm formation, motility, secretion, secondary metabolite production, and others” needs references.

L116-117: “strongly affected the transcription of crucial genes for the biosynthesis of c-di-GMP.”

L120: “higher concentrations of BHET.”

L122: Delete “for” after “encoding.”

L124: Write “concentrations of BHET” instead of “BHET concentrations.”

Suggestion for the legend of Figure 4: Remove the hyphen between “signaling related genes.”

L125: Replace “Further, we” with “We also.”

L126-135: Add the names of the genes in italics, add “of” after the displayed concentration of BHET. For example, “30 mM of BHET.” This is valid for the whole text.

Suggestion for the legend of Figure 5: Genes in italics; “*LuxP* is annotated to encode” and “*luxR* the high cell density regulator.”

L136: “Moreover, our data” instead of “further, our data.”

L136–138: Names of the genes in italics and parentheses.

L138: “levels on the presence of 30 mM of BHET.”

L139–141: merge the phrases “Finally, our data implied that BHET interfered with the cAMP-CRP signaling, as the catabolite activator protein transcription was almost completely turned off in the presence of 30 mM of BHET (Figure 6).”

L142: Replace “Besides” with “Alongside”

L143: write “it also strongly attenuated the transcription of motility genes”

L144: replace “In the contrary” with “On the contrary”

L150: “All of the three phages”

L151: add a comma and replace “In contrast” with “but”

L152: delete “However” and start with “In planktonic cultures, the supplementation with 30 mM of BHET”

Discussion:

L1: replace “Today” with “Hitherto”

L1–3: instead of “these enzymes have been characterized” write “and most of them were well-characterized regarding their structures, functions and catalytic activities on the synthetic polymer.”

L3–4: delete “and”, leaving “These enzymes are generally secreted promiscuous hydrolases belonging to E.C. 3.1.”

L4: replace “only a few studies” with “a limited number of studies”

L6: write “Despite these preliminary reports” instead of “first studies”

L7: replace “the different” with “distinct”

L9: Because “very” is informal, replace it with “a”, leading to “Our data suggest that only a few genes are differentially”

L10: replace “most likely” with “might not be able to differentiate between these two polymers”

L11: replace “the treatment with plasma” with “the air plasma treatment”

L13: delete “when grown” from “the most strongly transcribed genes when grown in biofilms (Table 2).”

L16: add “and establishment of life in the plastisphere.”

Suggestion for the Table 2 title: “The 25 genes with the highest normalized counts in DSM 21264 in PET and PE biofilms.”

L17-22: rewrite “For the natural polymers, such as cellulose, starch and chitin, it is well-known that the primary degradation products (dimers or monomers) are involved in activation of the pathways and transporters, being essential to the metabolization of the respective polymers. The activation of the genes involved in the catabolism of these respective monomers is subject to regulation by cAMP-CRP and, in part, c-di-GMP signaling. Thereby, especially chitin degradation is well-studied in *Vibrio* spp.”

L23: “little is known regarding PET and other synthetic polymers.”

L24-26: rewrite “positively controls the expression of the MHETase from the gram-negative *Idonella sakaiensis*, as well as the genes involved in TPA metabolism.”

L26: what do you mean by “this first study”? Is it another paper or your current manuscript?

L30: please reformulate. The operon encodes a protein that is involved in binding.

L31: “regulated by the presence of TPA”

L32-33: “However, they are both able to import and grow in the presence of TPA, using it as a single carbon and energy source.”

L36-38: “transcriptome datasets of DSM 21264, which gave us a deep insight into the metabolic activities of this bacterium in response to PET, BHET, TPA and distinct natural polymers.”

L38-39: the following phrase is in the wrong place “Because of the important role of *Vibrio* spp. in pathogenicity, regulatory networks have been well studied in this genus.”

L40-41: replace “all of them” with “the ones described.” There might be non-described *Vibrio* species that behave differently.

L41: replace “have” with “present”

L42-44: “at low levels under most environmental conditions tested (Figure 3),” It needs to be clearer that you mean the conditions tested in your work.

L44: replace “the transcription of the gene” with “gene transcription”

L47-53: I made small changes in these two phrases, but most importantly, they are lacking a reference. “These regulatory networks are known to be involved in several important processes in *Vibrio* spp., for instance, surface attachment, biofilm formation, infection, toxin and secondary metabolite production, and phage assembly. Biofilm formation in *Vibrio* spp. is controlled by an integrated and highly complex network of multiple transcriptional activators (e.g., VpsR, VpsT, and AphA) and repressors (HapR, H-NS); also, regulatory small RNAs and alternative RNA polymerase sigma factors are involved in the regulation of this process.”

L58: genes in italics

L60-62: please rewrite “the transcription levels of the autoinducer-I and 2 synthase genes, the cognate sensor genes, and the transcription of the periplasmic binding protein were also significantly affected by 30 mM of BHET (Figure 5).”

L68: replace “will most likely” with “would most likely”

L71: replace “Besides” with “Alongside this function,”

L76: replace “lifestyle” with “cells”

L79-80: “is involved in the metabolism of BHET”

L80-85: please rephrase to highlight your finding: “Until now, this novel function of UlaG was not described. The preliminary observation that the transcription of *ulaG* increased substantially in the presence of higher concentrations of BHET led us to perform experiments that confirmed this novel functional role of UlaG (Figure S3).”

L85: replace “Previous work has already” with “Previous studies demonstrated that”

L87-88: join two phrases together: “involved in the metabolism of EG, which was confirmed by both RNAseq and experimental data (Figure S5).”

L89: delete “already.” With “by the degradation of the polymer,” do you mean “by the release of degradation products from this polymer”?

L92: replace “and” with “as well as” after a comma

L96: replace “enriching for pathogens” with “involved in the enrichment of pathogens”

L97: replace “Finally” with “In conclusion”

Suggestion for Figure 7: “Figure 7. Possible PET degradation pathway in DSM 21264 and the regulatory model affecting QS, cAMP-CRP, and c-di-GMP signaling through BHET and TPA. Proteins

involved in enzymatic PET breakdown are displayed in cyan color. PET and the released degradation products are labeled in blue. Blue arrows indicate the regulatory pathways affected by BHET and/or TPA in DSM 21264. Red and blue arrows inside the cell model indicate the up- and downregulated pathways.”

Data availability: “under the accession number”

Materials and Methods:

L1-9: Why did you select two temperatures (either 22 or 28 °C), and when was each of them employed? AS medium is the artificial seawater medium that was supplemented with tryptone and yeast extract? If yes, then you can use the abbreviation ASW here and above (L2). Why did you perform additional growth experiments with M9 medium? What did you expect to see differently?

L12: replace “phenotypical” with “phenotypic”

L13-14: For the LB agar plates, you can say that the recipe was the same as mentioned above, with the addition of 15 g/L of agar. Otherwise, it is repetitive.

L15: “After autoclaving the medium”

L16: replace “the respective plate” with “in each plate”

L16-17: please rephrase “The bacterial incubation was performed using 5 µl of DSM 21264 (OD_{600 nm} = 1), 24 h at 28 °C.”

L18: “For the observation of biofilm formation on plastic surfaces, DSM 21264 ...”

L21-22: delete “shaking,” as it is already implicit with “80 rpm.” Please rephrase “After incubation, the foil was washed with PBS buffer and placed into a µ-slide eight-well”

L25-26: put all of the information together in the parenthesis: “(CLSM; Axio Observer.Z1/7 LSM 800 with airyscan; (Carl Zeiss Microscopy GmbH, Jena, Germany))”

L26-27: “C-Apochromat 63x/1.20W Korr UV VisIR objective” which manufacturer?

L28-29: please rephrase “For the image analysis, the ZEN software v.2.3 was used (Carl Zeiss Microscopy GmbH, Jena, Germany).”

L30: “Strains and polymers were incubated as described above” please indicate in which section this is described. Start a new phrase with “After incubation, they were fixed overnight”

L31: “1 % PFA in 50 mM Cacodylate buffer, pH 7” Before the abbreviation, add what is PFA. Add the recipe of the buffer.

L31: “On the following day, the samples...”

L32: what is GA? Also “For the appropriate drainage, the samples were incubated in 30, 50 and 70 %, respectively, for 20 min”

L33: “Drainage continued with 80, 96 and 100 % of ethanol”

L34: add the manufacturer of „Leica EM CPD300“

L35: replace “thereby washed 18 x” with “washing eighteen times”. Why specifically eighteen times?

L35-37: These lines need to be carefully rephrased and the manufacturer added

L40: add a comma after “To each well”

L41: replace “shaking at” with “and”

L41-43: “In order to get rid of planktonic cells, the foil was washed in PBS buffer and the cells were scratched off the surface”

L43: “stop mix (95 % ethanol and 5 % phenol)”

L44-45: “The supernatant was discarded and the pellet was washed three times in PBS buffer, being immediately”

L47: “artificial seawater” is ASW?

L47: “The respective source”. Here and above, when you mention PET and PE foil: add the manufacturer. The natural polymers also need the manufacturer to be added.

L50: add a comma before “as well as”. Delete “shaking”, as you already provide the revolutions per minute

L51-52: please rephrase “The negative controls included no additional carbon source and DMSO. The cells were harvested and pelleted”

L53: “resuspended in 800µl of RLT buffer”

L54: add a comma before “and cell lysis was performed using”. Add the manufacturer of the laboratory ball mill

L55: delete repeated information “(RNeasy Mini Kit Qiagen)”

L56: “96 % ethanol (v/v) was added”

L57-58: “the RNeasy Mini Kit was used as recommended by Qiagen, except for the exchange of RW1 buffer to RWT (also provided by the manufacturer), in order to isolate RNA fragments smaller than 200 nucleotides”. When you mention Qiagen, make sure to add the headquarters as well

L59: add a comma after “to determine the RNA integrity number,”

L61: “The remaining genomic DNA”

L62: “Thermo Fisher” without c. Is this the correct address of the headquarters?

L63: delete extra parenthesis

L64: “For the sequencing”

L66: What does “66” stand for?

L67: comma after “To assess the quality and size of the libraries”

L69: delete “(Agilent Technologies),” it is repetitive

L69: “The concentration of the libraries”

L74: replace “removing” with “removal”

L75: The reference “(Bolger et al., 2014)” needs to be in the right format. Also, correct “and a cut off was used”

L77: no need to put the program version numbers in parenthesis

L77-79: please correct this phrase “As mapping back-bone a file that contains all annotated transcripts excluding rRNA genes and the whole genome of the reference as decoy was prepared with a k-mer size of 11.”

L79: replace “done” with “performed”

L81: “as well as the sequence and position bias correction and adjustment to 10,000 bootstraps”

L82: “values of 325 and 25, respectively, were used”

L87: delete “sequences originating from”. Afterwards, the word “sequences” appears twice. Use “and low-quality sequences”

L89: reference and version of “BWA mem” and “DESeq2”

L90: I think “significantly differentially expressed” is confusing. Maybe “significantly expressed in a distinct manner (when compared to)” would be better?

L91: If you don't use the abbreviation FDR further, consider removing it. The same applies to abbreviations used less than three times. Also, add a comma after "or equal to 0.05."

L92-93: Replace "All software was used with standard parameters." with "Each software was used with default parameters."

L94: Replace "have been" with "was." If ENA is already written before, you can add just the abbreviation here.

L95: "under the accession number"

L97: Add the headquarters of "(Novagen/Merck)"

L98: "using the restriction sites." Replace "in front of the" with "located upstream of the C-terminal His-6-tag."

L100: "by the design tool of the program"

L101: "7 μ L of DNA was mixed." Indicate the manufacturer of T4 ligase and buffer.

L102: Replace "purified" with "pure" and write "overnight" together.

L103: "and positive clones were identified by Sanger sequencing." Add the headquarters of Microsynth seqlab.

Suggestion for the subtitle: "Recombinant protein expression and purification"

L104-108: Start with "The proteins were overexpressed by growing E. coli T7 Shuffle harboring pET21a(+):*ulaG*, or BL21(DE3) harboring pET28a(+):*pet6*, at 37 °C to $OD_{600nm} = 0.8$. The protein expression was induced with 0.4 mM of IPTG and incubated at 22 °C overnight, until cells were harvested by centrifugation at 5,000 g." Fully write IPTG the first time this abbreviation appears, add the manufacturer of the centrifuge, and check if the unit given for the centrifugation is in g within the text.

L109-111: "For the protein purification, the cell pellet." Write appropriately the manufacturer of the sonicator.

L111: "with an amplitude of 70 % and a cycle of 0.5." I don't understand this cycle of 0.5; is there a measurement unit?

L112: Replace "six-fold" by "C-terminal His-6-tag." Make sure that, when you want to write on the singular, it is written "C-terminus," but in this case, "C-terminal" is correct.

L113: “potassium phosphate buffer 0.1 mM”

Suggestion for the subtitle: “Quantification of PET and BHET degradation”

L115: “of M9 medium”

L116: Delete “additional,” leaving “and 5 mM of BHET.”

L116: “The flasks were incubated at 28 °C”

L117: Add a comma after “Each day.” Replace “taken” with “collected” or “aliquoted.”

L118-119: “As a negative control, the medium was incubated under the same conditions with *E. coli* Dh5 α without the vector, and samples were taken at the same time points.”

L120: Add a comma after “foil degradation.”

L121-122: “6-well plates. Besides, 0.5 mL of the sample was taken each day and”

L123: “For the enzymatic BHET degradation, pure enzymes in varied concentrations”

L125: Replace “Respective” with “The.”

L127: “The promoter of”

L128: “softberry.com (BPROM - Prediction of bacterial promoters)” Make sure to add the date of accession and if it is available online.

L129: “downstream of the respective gene”

L130: “using the restriction enzymes”

L131-132: “The steps for the primer design.” Rather than writing “described above,” indicate the section.

L132: Replace “Positive constructs” with “The constructs with expected DNA size.”

L133: Replace “Once” with “Since.”

L134: Replace “colony amount” with “colonies.” How many colonies approximately did you inoculate in each culture?

L135: Replace “Cells were grown in overnight cultures.” with “and the cells were grown overnight.”

L136-137: “1 mL of the donor (*E. coli* WM3064 carrying the respective plasmid) and the receptor strain (DSM 21264) were mixed at a ratio of 1:1.”

L137: “and centrifuged at 5,000 rpm for 8 min.” Please add centrifugation in g.

L138-140: “The cells were washed (...), resuspended in 150 µl of the same medium after the final centrifugation step, and spotted onto LB agar supplemented with“

L140: “and the spot was washed off“

L141-142: “ASW agar plates supplemented with 25 µg of chloramphenicol and 25 µg of kanamycin.“

Did you use both antimicrobials simultaneously?

L142-143: “The colonies could be detected“

L145: replace “For the identification of“ with “To investigate“

L146-147: “carrying the promoter fusion construct pBBR1MCS-1::*adhE::amcyan*, as well as the wild type strain, were (...) in M9 medium supplemented with 5 mM of glucose“

L149: delete “shaking“

L49-151: I suggest rewriting the phrase as follows: “To detect the influence of EG, 5 mM of this compound was added, and the negative control included strains growing in the absence of EG.“

L152-154: “the optical density and fluorescence of cyan (excitation 450 and emission 496) (...) at the Synergy plate reader using the Gen5 software (...). The average was measured, and the fluorescence was divided by the optical density in order to detect fluorescent units“

Suggestion of subtitle: Air plasma activation of PE and PET foil

L155: “For the plasma activation“

L158: add a comma before “for an expanded treatment width“

L160: replace “for repetitious accuracy“ with “to replicate accuracy regarding the treatment conditions“

Still in the topic of air plasma activation: make sure that the manufacturers are cited.

I spotted a typo in the **acknowledgements** section: please correct to “and supported by“

Declarations

Potential competing interests: No potential competing interests to declare.