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Method for acrylic acid monomer detection with recombinant biosensor cells for enhanced plastic degradation monitoring from water environments

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ABSTRACT

Plastic debris degrades in the water environments due to various factors such as mechanical stress. Small-sized degradation products, including plastic monomers, are currently monitored using equipment which might be unsuitable for screening. Here, we developed a recombinant whole-cell bacterial biosensor, which could be used for this type of monitoring. The *Escherichia coli* pBAV1K-ACU-lucFF cells contain a luciferase-based reporter system under the control of acrylic acid specific promoter. The biosensor cells were used to detect acrylic acid monomers from both sterile water and spiked lake water samples, indicating usability with environmental samples. Furthermore, poly(acrylic acid) was incubated in salt water, and the biosensor cells could identify acrylic acid monomers originating from it. Thus, the cells could be used to observe similar processes in the environment. The results show that the bacterial biosensors could complement the current research methods of plastic monomer monitoring in water environments with a potential for higher throughputs.

1. Introduction

Plastics and their degradation products can be detected from marine and freshwater environments (Guo and Wang, 2019; Li et al., 2018) and land-locked water bodies, such as ponds in industrial areas (Liu et al., 2019). As human originated plastic debris degrades in the environment due to biotic or abiotic factors, such as ultraviolet irradiation or mechanical stress (Zhang et al., 2021), different sized particles are formed. The environmental effects and fate of the degradation products are widely studied, and they depend on the properties of the particles (Li et al., 2018). Mechanical stress is an especially important factor in the degradation of synthetic fibers, such as acrylic, which forms 2.4% of the plastic particles in a Chinese mangrove sediment (Zhang et al., 2021; Zhou et al., 2020). Currently, plastics and their degradation products can be studied for example by using micro-Fourier Transformed Infrared Spectroscopy (μ-FTIR). There is a size limit for the μ-FTIR identification (Frias and Nash, 2019), and as the plastics disintegrate (Zhu et al., 2020), plastic monomers below this size limit could be released and remain undetected.

Recombinant whole cell bacterial biosensors (further referred here to only as biosensors) could be used to monitor the degradation or disintegration of plastic polymers into their monomers, as well as the further mineralization or assimilation (Zhang et al., 2021) of the monomers. The biosensor cells can include genetic elements from two or more species for specific detection of target compounds and are suitable for high-throughput testing, which reduces costs. The biosensors are based on reporters, such as light emitting luciferases. (Karp and Galluzzi, 2006). The reporter gene can also be subjected to the control of a substance specific promoter. This allows specific detection of the substance, creating a "lights on" biosensor cell. (Belkin, 2003). Because the substance must enter the cell to interact with the promoter, the "lights on" cells measure both bioavailability and quantity of the substance.

Whole cell bacterial biosensors have previously been developed for acrylic acid (AA) (Meyer et al., 2019), but to our knowledge have not been used for the context of water environment monomer monitoring. In this study, a bioluminescent bacterial biosensor was developed for AA which is a monomer of a plastic, poly(acrylic acid), PAA. We constructed a "lights on" biosensor cell based on firefly luciferase (*lucFF*). We demonstrated that it detects and quantifies AA from spiked lake water samples. Furthermore, the developed biosensor can recognize AA monomers originating from PAA incubated in saline water.

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2. Materials and methods

2.1. Bacterial strains and plasmids

The plasmid pAJM.884 was a kind gift from Christopher Voigt (Addgene #108536) and pBAV1K-T5-GFP (Addgene #26702) from Ichiro Matsumura. Plasmid pBestLuc was supplied by Promega, USA. All plasmids were maintained in *Escherichia coli*. Toxicity biosensor strain contained pCGLS11 plasmid (Vesterlund et al., 2004).

2.2. Cultivation

All bacteria were cultivated (16 h, 30 °C, 300 rpm) in lysogeny broth (LB) medium containing 10 g/L tryptone (Labema, Finland), 5 g/L yeast extract (Labema, Finland) and 10 g/L NaCl (VWR, USA). For liquid *E. coli* cultivations, the LB was supplemented with 0.1 M phosphate buffer (pH 7; final concentrations of K_2HPO_4 9.3 g/L (Merck, USA) and KH_2PO_4 6.3 g/L (VWR International, USA)). The lysogeny agar (LA) plates were cast from LB medium supplemented with 7.5 g/L of agar (SigmaAldrich, USA) and 1% glucose (Merck, Germany). Both LB and LA were supplemented with suitable antibiotics to maintain the plasmids. Kanamycin (50 μg/mL; Janssen, USA) was used for pBAV1K-T5-GFP, pAJM.884 and the constructed plasmids (pBAV1K-ACU-lucFF, pBAV1K-ACU-GFP), while ampicillin was used for pBestLuc and toxicity biosensor *E. coli* K12 pcGLS11 (100 μg/mL; SigmaAldrich, USA).

2.3. Biosensor construction

Molecular biology reagents, such as buffers, primers, and enzymes, were purchased from ThermoScientific, USA. GeneJet PCR purification and gel purification kits were purchased from Fermentas, USA. USER enzyme was supplied by New England BioLabs, USA.

First, the acrylic acid sensor elements were amplified with polymerase chain reaction (PCR) from pAJM.884 using primers FW_acu and RV_acu (Table 1). The resulting fragment and pBAV1K-T5-GFP were digested using *Xba*I and *Spe*I, purified with gel electrophoresis and combined. The resulting plasmid (pBAV1K-ACU-GFP, Supplementary Fig. 1) was electroporated into *E. coli* XL1 Blue.

Next, the biosensor plasmid for acrylic acid was constructed using USER (uracil specific excision reagent) cloning (Geu-Flores et al., 2007). Briefly, pBAV1K-ACU-GFP was PCR amplified with FW_bb and RV_bb, and pBestLuc was amplified using FW_lucFF and RV_lucFF primers to obtain the plasmid backbone and the *lucFF* gene (Supplementary Fig. 1). These fragments were *Dpn*I digested, joined with USER and ligated with T4 ligase. The resulting plasmid was electroporated into *E. coli* XL1 Blue and cultivated on selective LA plates to select the correct plasmid. The developed plasmids are available upon request from the authors.

2.4. Samples

AA was diluted into double distilled water (DDW) or lake water. The lake water was collected on February 4th, 2020 from the shore of a freshwater lake in Western Finland (61°31′30.9″N 23°42′37.5″E) and frozen until usage. All samples were handled in polypropylene tubes or dishes. The final test concentrations were 1, 10, 100, 1000, 10 000 and 100000 μM. For cross-reactivity tests, similar concentrations MMA (methyl-methacrylate) and PA (propionic acid) were used.

For the polymer incubation tests, 100 mL of 3.5% NaCl in DDW was placed into a glass bottle to simulate marine environment. Then, 2.16 mL of DDW was added to PAA (0.72 g) or PMMA (poly-(methyl-methacrylate), 1.00 g), mixed with a metal spatula and incubated for 5 min to allow PAA hydrogel formation. The PAA hydrogel or the PMMA-DDW mixture were then added to separate glass bottles and stirred on a magnetic stirrer (Stuart Equipment, UK) for 6 h at 100 rpm. Samples (700 μL) were collected into glass vials immediately after adding the polymers and then every 2 h for 6 h. All monomers and polymers were purchased from SigmaAldrich, USA.

Table 1
Primers used in this study.

Primer	Nucleotide sequence
FW_acu	GCC GAC TCT AGA CCA ATT ATT GAA GGC CTC C
RV_acu	GCC GCT ACT AG T CAG ATA AAA TAT TTG CTC ATG AGC
FW_bb	AAC TCG GUA CCA AAT TCC AGA AAA GA
RV_bb	ATC TAG UAT TTC CCC TCT TTC TCT AGT
FW_lucFF	ACT AGA UGG AAG ACG CCA AAA ACA T
RV_lucFF	ACC GAG TUA CAA TTT GGA CTT TCC GC

2.5. Assay protocol

An aliquot of 50 μL of the samples was pipetted in triplicate into the wells of a white, opaque 96-well polystyrene plate (Corning, USA). The over-night cultivated biosensor cells (2.0 optical disturbance at 600 nm) were diluted 1:1 with fresh LB, and 100 μL of this mixture was added into the wells. The plate was incubated in a plate shaker (400 rpm, 30 °C, 1 h). Next, 100 μL of 1 mM D-luciferin (Biothema, Sweden) in 0.1 M citrate buffer (pH 5; final concentrations of sodium citrate dihydrate 17.1 g/L and citric acid 8.04 g/L; both supplied by Merck, Germany) was added into the wells. Bioluminescence was measured immediately after the addition of D-luciferin with Chameleon Multi-label microplate reader (Hidex Oy, Finland). For fluorescence measurements, no D-luciferin was added, and the plate was measured with Fluoroskan (ThermoFisher, USA). To determine the signal-to-noise ratio for the bioluminescent biosensor cells, the bioluminescence of three wells containing LB only was measured in two test runs for the background noise signal. A graphical representation of the assay protocol can be found in Supplementary Fig. 2.

The results are expressed as IF (induction factor). IF is the fold change calculated from the averages of the three sample wells and the zero control wells (samples contained only DDW, lake water or 0 h polymer degradation sample). The toxicity test was performed as earlier described (Poikulainen et al., 2020). Briefly, AA samples were incubated with the *E. coli* psGLS11 biosensor cells (optical disturbance 2.0, in LB) for 60 min, and their luminescence was monitored using the Chameleon Multi-label microplate reader. An inhibition percentage (−%) was calculated.

3. Results and discussion

3.1. Development of E. coli pBAV1K-ACU-lucFF

At first, the toxicity of AA for *E. coli* was tested. AA samples were incubated with *E. coli* pCGLS11 (Fig. 1A), and the inhibition percentages were 11.0% for 10 000 μM and 99.7% for 100000 μM. Therefore, the highest tested concentration ceased nearly all the metabolic activity of the *E. coli* cells. This is in accordance with previous studies, which have suggested that the AA might be toxic due to its acidity and possible participation in glutathione depletion (Straathof et al., 2005). The developed biosensor should only be used for measurements at lower, less toxic, concentrations to assure proper function. However, AA is present in sea waters in nanomolar concentrations (Vairavamurthy et al., 1986), and thus the environmental samples should not prevent the usage of these biosensor cells. If more robust biosensor cells would be needed in the future, the developed sensor plasmid could be transformed into another species. There are a number of bacterial species, which are able to biodegrade acrylic polymers (Gaytán et al., 2021), and thus it could be assumed that a more tolerant host could be found.

Next, the *E. coli* pBAV1K-ACU-GFP strain was tested for the detection of acrylic acid monomers. The induction was proportional to the concentration of AA, but the highest recorded IF, at 10 000 μM, was only 1.02 (Fig. 1B). Thus, we decided to optimize the biosensor cells by using

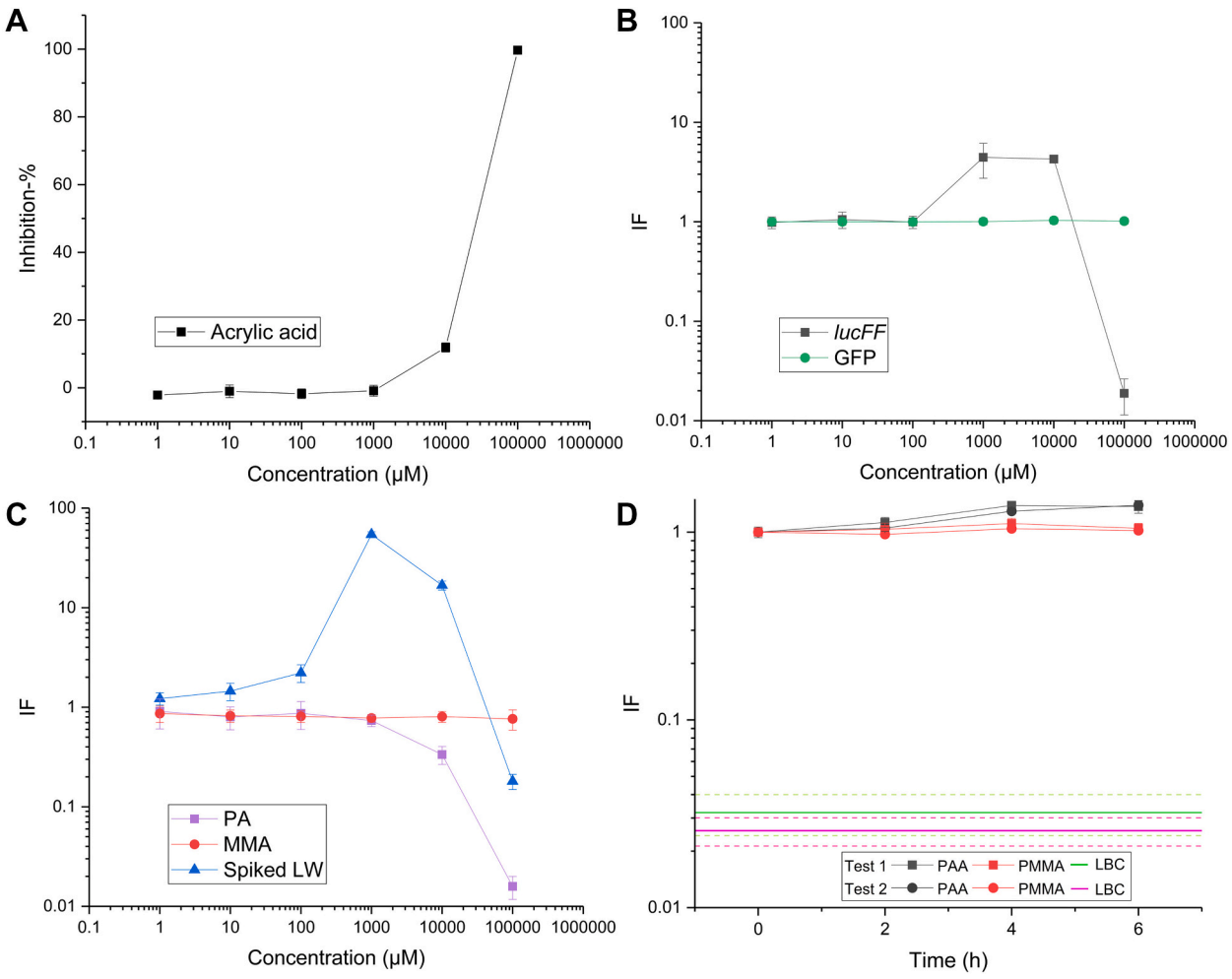

Fig. 1. Note logarithmic scales on X axes of A–C as well as the logarithmic scales on Y axes of B–D. Error bars represent coefficient of variation (CV-%). A. Toxicity test showing inhibition percentages (−%) caused by acrylic acid when incubated with *E. coli* pCGLS11. B. The average IF (induction factors) caused by acrylic acid when incubated with *E. coli* pBAVA1K-ACU-GFP (GFP) and *E. coli* pBAV1K-ACU-lucFF (lucFF) in three biological replicate test runs. C. The average IF caused propionic acid (PA), methyl-methacrylate (MMA) or by lake water (LW) spiked with acrylic acid when incubated with *E. coli* pBAV1K-ACU-lucFF in three biological replicate test runs. D. The IF caused by salt-water incubated poly(acrylic acid) (PAA) and poly(methyl-methacrylate) (PMMA) samples when incubated with *E. coli* pBAV1K-ACU-lucFF in two biological replicate test runs. LBC is the IF of wells containing only culture media, dashed lines represent its CV-%.

more sensitive reporter system. Hakkila et al. (2002) have previously concluded that luciferase reporter systems, such as the *lucFF*, have faster and more sensitive responses than the fluorescence reporters, such as GFP. Therefore, the *E. coli* pBAV1K-ACU-lucFF was developed for higher signal levels and lower limit of detection. This is illustrated in Fig. 1B, where the IF of the *lucFF* sensor is approximately as high (1.03) at 100 μM, as the GFP sensor had at 10 000 μM. The highest IFs were recorded at 1000 and 10 000 μM of AA. The signal level decrease at higher concentrations is likely caused by the toxicity of AA for the cells, as discussed above.

There are chemicals similar in their chemical structures compared to AA, such as methyl-methacrylate, monomer of poly(methyl-methacrylate), and propionic acid, monomer used in many *co*-polymers (Danner et al., 1998; Greim et al., 1995). To confirm, that the induction caused by AA in the biosensor cells is specific, PA and MMA were tested in three biological replicate test runs. The average IF, of these three tests, for PA was 0.33 and for MMA 0.80 at 10 000 μM (Fig. 1C). In result, no induction for either potential cross-reactor was detected at or below 10 000 μM. At higher concentrations PA and MMA were deemed toxic for the biosensor cells, as illustrated by the ceasing of light production (IFs ≪ 1 in Fig. 1C).

As discussed above, AA is present in seawater at nanomolar concentrations. However, propionate, an ion of PA, can be produced by anaerobic bacteria in marine sediments (Mueller-Harvey and John Parkes, 1987). Yet, the concentration in the sediment was measured to be less than 24 μM (Sansone and Martens, 1982). Similarly, the concentration of MMA in industrial wastewater is lower than 160 μM (Almazán-Sánchez et al., 2014). Thus, it can be deduced that the potential concentrations of the cross reactors in environmental samples should not interfere with the function of the developed biosensor cells. The bioluminescent signal was deemed specific for only AA monomers.

The intended use of the *E. coli* pBAV1K-ACU-lucFF biosensor cells will be the monitoring of AA monomers and their bioavailability from environmental samples. Accordingly, it was confirmed that they can detect AA from lake water as well as DDW. The lake water was spiked with AA and tested in three biological replicates with three technical replicates in each test run. As the results show (Fig. 1C), the biosensor cells were well able to detect AA from the spiked lake water. The IFs were dose dependent between 1 and 1000 μM, and even the slightly toxic 10 000 μM still showed an IF of 16.8. The lake water tests also had the overall highest average IF levels (Fig. 1B–D), indicating that the lake water is a suitable sample material for the biosensor cells. The high levels could also indicate that the lake water samples contain nutrients that boost the function of the biosensor cells compared to the sterile DDW.

3.2. Aging of the polymers

Poly(acrylic acid) and its *co*-polymers are widely used in consumer products, and $1.58 \cdot 10^9$ kg of PAA was produced in 2008 alone (Wiley-VCH, 2016). For example, PAA can be used in cosmetic and personal care products (Gaytán et al., 2021; Somasundaran et al., 2004). As the consumers use these products, PAA can reach lakes or seas through the sewer and water treatment systems. Ultraviolet light (Prajapat and Gogate, 2016) and mechanical stress (Gaytán et al., 2021; Zhang et al., 2021) from the environment, such as the waves, degrade the PAA. To simulate this type of degradation, PAA was exposed to mechanical stress from stirring in 3.5% NaCl water solution, a typical salt concentration of sea water (Wetzel, 2001).

The *E. coli* pBAV1K-ACU-lucFF biosensor cells detected AA monomers originating from the PAA during the first 6 h of incubation under mechanical stress in two biological replicate test runs (Fig. 1D). At 6 h, both test signals were statistically higher than the signal of zero-hour sample ($p < 0.01$, for each test run, in Student's unpaired *t*-test). It has been previously shown that even 1 h of exposure mechanical stress from ultrasonic treatment can cause depolymerization of PAA (Prajapat and Gogate, 2016). Thus, it is fair to assume that 6 h of mechanical stress can start some disintegration of the polymer. In addition, when a related polymer, PMMA, was exposed to similar treatment, no induction could be detected with the cells (Fig. 1D). The PMMA signals at 6 h were not statistically different to those of the zero-hour sample ($p = 0.41$ and $p = 0.76$, for Test 1 and Test 2 respectively). This indicates that the light production of the sensor cells is caused specifically by AA monomers originating from the PAA, and not by a related polymer. Furthermore, the increased NaCl concentration caused by the aging samples did not interfere with the function of the biosensor cells. This suggests that the cells could be used to measure AA monomers from seawater samples in addition to the lake water samples.

The average IFs of PAA and PMMA were also compared to the average background noise signal caused by the cultivation medium only (LBC, Fig. 1D). Even though the IFs caused by the disintegration products of PAA remained lower compared to the results from AA monomers (Fig. 1B), it was still evident, that the biosensor cells produced more bioluminescent light than the background sample, equaling to a signal-to-noise ratio of 39. Thus, it can be concluded, that PAA released AA monomers during the 6 h, and that the *E. coli* pBAV1K-ACU-lucFF detected the resulting monomers. In addition, the monomers had to be bioavailable to be detected.

4. Conclusion

Studying the degradation products of microplastics in water environments often requires either vast and skilled personnel resources or high valued investments in specialized equipment. In addition, many of the current methods are not suitable for screening purposes. In this article, we have developed a recombinant whole cell bacterial biosensor using *Escherichia coli* as the host organism to complement the current research methods. We have also demonstrated that the bioluminescent biosensor cells can distinguish degradation products, which have been detached from PAA in salt water. This technique has potential for high-throughput screening, as the testing is fast to perform in a 96-well plate format. The technique could be further developed to have even higher signal-to-noise ratio either by enhancing the sensitivity of the transcription factor or by improving tolerance of the cells toward the toxic effects AA by transforming the plasmid into another, more tolerant bacterial species. Finally, the technique could be expanded for other monomers by re-designing the sensor elements.

CRediT authorship contribution statement

Emmi Puhakka: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Ville Santala:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

I declare that we do not have any conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2022.113568.

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