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Whole-Cell Paper Strip Biosensors to Semi-quantify Tetracycline Antibiotics in Environmental Matrices

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ABSTRACT

A novel, low-cost, and portable paper strip biosensor was developed for the detection of tetracycline antibiotics. *Escherichia coli*/pMTLacZ containing the tetracycline-mediated regulatory gene used as recognition elements with β-galactosidase as the reporter protein was designed and applied to cheap and portable Whatman filter paper as the carrier to prepare this paper strip biosensor. The detection process was optimized by using EDTA and polymyxin B as a sensitizer to improve the accuracy of detection for complicated matrices. The paper strip biosensor was suitable for tetracycline concentrations in the range of 75–10000 μg/L in water and 75–7500 μg/L in soil extracts. Detection limits of 5.23–17.1 μg/L for water and 5.21–35.3 μg/kg for the EDTA soil extracts were achieved at a response time of 90 min. The standard deviation (SD) of detected values by the biosensor paper strip compared to those determined by HPLC was between 13.4−59.6% for tetracycline and 2.01−33.5% for oxytetracycline in water and was between 6.22−72.8% for tetracycline and 5.90−43.4% for oxytetracycline in soil. This suggests that the paper strip biosensor was suitable for detecting both tetracycline and oxytetracycline in water, and could provide a suitable detection for extractable oxytetracycline in soils. Therefore, this biosensor provides a simple, economical, and portable piece of field kit for on-site monitoring of tetracyclines in a variety of environmental samples, such as pond water and agricultural soil that are susceptible to tetracycline pollution from feed additives and fertilization with livestock manure.

Keywords: Paper strip; Tetracyclines; Detection; Semi-quantity; Water; Soil

1. Introduction

Antibiotics are largely used in human medicine, animal husbandry, agriculture, and aquaculture (Hoa et al. 2011; Teuber 2001). The rampant usage of antibiotics has led to their ubiquitous occurrence in environmental compartments, including water, soil, and sediment impacted by wastewater, sewage sludge, or livestock manure, etc (Berendonk et al. 2015; Gothwal and Shashidhar 2015; Liu et al. 2017). Until now, over 30 types of antibiotics, including those from the tetracycline, sulfonamide, macrolide, and quinolone classes of antibiotics, have been detected with concentrations typically at microgram per liter levels in pond waters (Limbu et al. 2018; Liu et al. 2017; Rico et al. 2017) and microgram per kilogram levels in soils and sediment (Hu et al. 2010; Kumar et al. 2005; Liu et al. 2009). However, in heavily impacted agricultural waters, concentrations have been measured at the milligram per liter level (Bartelt-Hunt et al. 2011; Peak et al. 2007; Zilles et al. 2005). One major concern is that environmental exposure to these antibiotics can induce resistance in native bacteria, contributing to the development of the environmental resistome, and resulting in lower effectiveness of antibiotics in the treatment of bacterial infections.

Preventing the environmental introduction of antibiotics is an ideal way to reduce the proliferation of antibiotic resistant bacteria and antibiotic resistance genes (Berendonk et al. 2015; Gao et al. 2018; Rodriguez-Mozaz et al. 2015; Zhu et al. 2013). Among the antibiotics, tetracyclines are essential in modern intensive agriculture production and widely used in livestock and mariculture farming (Gu et al. 2020; Scarano et al. 2018). Currently, there is a growing effort to reduce their input and impact (Du and Liu 2012; Hu et al. 2010; Kumar et al. 2005; Liu et al. 2017; Thiele-Bruhn 2003). Quick, easy, and cost-effective methods are urgently needed to monitor and support the management practices for drug control during food production as well as following their introduction

into water and soil.

Currently, numerous antibiotic detection methods have been developed, most of which being chemical analytical methods utilizing high-performance liquid chromatography and mass spectrometry for the extraction and separation of antibiotics from complex environmental samples. These traditional methods require complicated, time-consuming, reagent heavy processing and expensive instrumentation that relies upon the user's expertise to interpret results (Batt and Aga 2005; Hamscher et al. 2002). Recently, paper test strips have been developed for the detection of bioactive contaminants like antibiotics. Most of these paper sensors are based on aptamers or monoclonal antibodies as recognition elements with nanofibers or gold nanoparticles acting as transducers (Abbas et al. 2013; Ferreira et al. 2015; Liana et al. 2012; Ornatska et al. 2011). Nevertheless, some deficiencies of these sensors have been recognized and include time-consuming, expensive, cumbersome fabrication (Gullapalli et al. 2010) or insufficient sensitivity due to shifting ion level, pH, temperature, or light interference (Ahmed et al. 2014; Chaiyo et al. 2015; Hossain et al. 2009; Quesada-González and Merkoçi 2015). All these factors have impeded the practical application of existing sensors for antibiotic detection in soils and water.

Whole-cell biosensors provide a self-contained portable sensing system based on genetically engineered whole cells that physically are adsorbed on filter-paper strips for on-site semiquantitative visual monitoring of N-acylhomoserine lactones (AHLs) agonists in a test sample (Struss et al. 2010). This paper strip biosensor could serve as a simple and economical portable piece of field kit for on-site monitoring of AHLs in various types of environmental samples. So far, the efficacy of these antibiotic biosensors have only been demonstrated in preliminary research for the detection of antibiotics in water and soils under laboratory conditions (Ma et al. 2020). Studies on antibiotic colorimetric strips

using whole-cell biosensors for on-site semiquantitative visual testing are not available.

In this study, we reported the development of a self-contained sensing system deployed on a paper strip for the detection of tetracyclines in environmental samples. This sensing system was based on genetically engineered bacterial cells that were directly dried on filter paper strips. These bacterial sensing cells employ β-galactosidase as the reporter protein, which can serve visual detections for antibiotics by using a chromogenic enzyme substrate (X-gal). The sensing system was validated by application in the detection of tetracyclines in water and soil. Paper strip biosensors allowed for visual, fast, convenient, and dose-dependent monitoring of tetracyclines in tested samples, thus demonstrating their value as a portable tool for on-site analysis of environmental samples.

2. Methods

2.1 Chemicals

All chemicals used in this study are described in Supplemental Text S1 and Table S1 of Supporting Information (SI).

2.2 Plasmids construction

The gene sequences corresponding to the pMT fragment from the tetracycline-mediated regulatory system of *Staphylococcus rostri* strain RST11:Tn*916* and transposon Tn*10* were isolated from plasmid pMTmCherry using polymerase chain reaction (PCR) with primers (Table S2, Table S3). The *lac*Z fragment encoding β-galactosidase was PCR-amplified by the primers (Table S3) from the pUC19 plasmid (Table S2). The pMTLacZ plasmid was prepared by recombining the *lac*Z fragment and pMT fragment using TreliefTM SoSoo cloning kit. The recombined plasmids were subsequently transformed into competent *Escherichia coli* BL21 cells (Table S2) according to molecular cloning protocols and then verified using highly specific primers

(Table S4) (Sambrook et al. 1989). The transformed cells were sifted via culturing on Lysogeny broth (LB) agar plates containing 100 mg/L ampicillin and 20 mg/L X-gal at 37 °C overnight for screening *E. coli* BL21/pMTLacZ.

2.3 Fabrication of paper strips

Paper strips were prepared as described by Struss *et al*. with some modifications (Struss et al. 2010). Biosensor cells were cultured for about 4−5 hours at 37 °C and at 150 r/min in the LB medium (containing 100 mg/L ampicillin), resulting in an OD_{600} of 0.450−0.500. Cells were centrifuged at 4,000 × *g* for 10 min and resuspended in 1/4 lysogeny broth with 10% lactose and polymyxin B. The biosensor cell suspension (50 µL) was spotted on Whatman filter paper strips (1 × 4 cm), dried for 10 min at room temperature and then subsequently dried by vacuum freeze-drying. These paper strips were stored at -20 °C for further study.

2.4 Tetracycline analysis in water

Standard solutions of six tetracyclines were prepared individually at fourteen concentration levels (0, 10, 25, 50, 75, 100, 250, 500, 750, 1000, 2500, 5000, 7500 and 10000 µg/L) in sterilized Milli-Q water. Each tetracycline solution (100 µL) or environmental water sample was mixed with 900 µL LB medium (containing 100 µg/mL ampicillin) in a polyethylene tube. Previously prepared paper strips were inserted in these culture tubes and incubated at 37 °C without shaking for 90 min. Paper strips were then taken out of the culture tubes and prevented from drying. After, 10 µL of X-gal substrate solution (50 g/L) in DMF was added on the biosensor cell spot. The paper strip was shielded from light at 37 °C for 90 min for color development. A Sony α7 III digital camera (Sony, Tokyo, Japan) with Sony shots (20 mm F1.8, Tokyo, Japan) was used for taking RAW images of the strips (Struss et al. 2010).

The color intensities, rather than the size of the blue area, an artifact of differences in

147 the dispersive size of the X-gal color developing agent, were measured using the software
148 ImageJ (National Institutes of Health, Bethesda, Maryland, US)
149 (https://imagej.net/Downloads) upon acquired digital images above and used to determine
150 tetracycline concentrations. The measurement settings of the images were set to mean gray
151 value in ImageJ that converts 100% white as 255 and 100% black as zero. A rectangle
152 section (1 × 1 mm area) in each image was drawn around a spot on the strip and measured
153 using the selection tool in ImageJ software. A background measurement was implemented
154 of the same size on the bare paper strip, to normalize for slightly different color intensities
155 due to changing illumination while these pictures were obtained in the field. The paper
156 strip biosensor-based calibration was confirmed with the tetracycline concentration
157 quantified by high-performance liquid chromatography (HPLC) (Fig. S1). The limit of
158 detection (LOD) of a biosensor is usually calculated as the concentration at which
159 biosensor signal to noise ratio is above 3. Therefore, while measuring the pixel density
160 changes in samples devoid of tetracyclines (buffer only), biosensor noise was determined
161 and used for the calculation of the LOD for the paper strip biosensor (S/N=3). The
162 linearity ranges of the biosensors paper strip were calculated according to the r values of
163 the standard curves greater than 0.800 (Burrows and Watson 2015). The obtained standard
164 curves were then used for analyses of tetracyclines in water samples ($W_{1\text{-}20}$).
165 Twenty water samples were obtained from Nanjing fishponds (Physicochemical
166 properties of the water samples are provided in Table S5 of SI). The sampling sites of
167 water samples were labeled on the map of Nanjing and these samples were mainly
168 collected around Xuanwu lake and Yueya lake of Nanjing (Fig. S4). A random dose of
169 either tetracycline ($W_{1\text{-}10}$) and oxytetracycline ($W_{10\text{-}20}$) was added to the twenty water
170 samples (W_1−W_{20}), which was used to simulate polluted pond water from the input of feed
171 additives used in aquaculture. These samples were aged for 30 days under outdoor

conditions prior to analysis. HPLC (LC-20AT, Shimadzu Co., Kyoto, Japan, Detailed process in SI of Fig. S2) was first used to screen and quantify tetracycline concentrations. The estimated concentration of tetracyclines was detected by paper strip and calculated using the fourteen-point paper strip calibration described above.

2.5 Tetracyclines analysis in soil

A volume of 100 µL of each of the soil matrix calibrants (see Supplemental Text S2 for the preparation process) was added to 900 µL of LB medium in culture tubes in triplicate. The detection procedure was performed as described for water. A dose-response curve using standard tetracyclines solutions prepared in soil extracts was obtained by paper strip biosensors in each analytical run as well as HPLC as a reference for comparison.

Twenty tetracycline-contaminated Inceptisol samples were obtained from a test field at Nanjing Agricultural University (Nanjing, China). The physicochemical properties of Inceptisol are given in Table S6. Ten soil samples ($S_{1\text{-}10}$) were obtained from the tetracycline test field which received tetracycline exposures for at least one year, and ten soil samples ($S_{11\text{-}20}$) were obtained from another test field that was contaminated with oxytetracycline for at least one year. Each processed soil extract (100 µL) (i.e., the EDTA soil extract, see Supplemental Text S3 for the pretreatment process of soils) was analyzed by the methods described previously using the paper strip biosensor. The concentration was then determined by colorimetrics based on the matrix matched calibration curve described above.

3. Results and discussion

3.1 Biosensor construction and paper strip production

The schematic of pMTLacZ plasmids used in this study is shown in Fig. 1A. The *lac*Z

197 gene and T7 gene were fused to obtain *lac*Z-T7 gene (Fig. 1B). To construct this biosensor
198 for the sensing of tetracyclines, the pMT gene (Fig. 1B), *lac*Z-T7 gene (Fig. 1B) and T7
199 gene (Fig. 1B) were used to construct the biosensor plasmids by DNA homologous
200 recombination. Additionally, the recombinant plasmid was further verified by PCR with
201 specific primers, and the amplicon lengths were consistent with those expected (pMTLacZ
202 3590 bp) (Fig. 1C). As shown in Fig. 1a, *E. coli* BL21 strain containing a pMTLacZ
203 plasmid produced the blue colony with X-gal when exposed to tetracycline. The
204 expression of the reporter gene is under tight transcriptional control of the tetracycline
205 repressor (*tet*R) on the plasmid (pMTLacZ, Fig. 1A and Fig. 1D). Binding of tetracycline
206 to *tet*R abolishes the binding of this gene to two operator sites (*tet*O) and thus allows
207 expression of the *lac*Z gene (Fig. 1D) and translation of β-galactosidase (Orth et al. 2000).
208 X-gal can be degraded by β-galactosidase, which then produces the blue signal
209 (5,5`-dibromo-4,4`-dichloro-indigo, Fig. 1E). The induction results of tetracyclines
210 revealed that exposure to an increased concentration of antibiotic can increase the enzyme
211 activity response of the biosensor with a dose-related effect (Fig. S3, Detailed illustration
212 in SI).

213 Based on this character of the constructed bacterium, the paper strip biosensor was
214 designed as follows (Fig. 1F). The biosensor cells were cultured in the LB medium,
215 centrifuged and resuspended in 1/4 LB with 10% lactose and PMB (Fig. 1F, step i-iii). The
216 suspension was spotted on Whatman filter paper strips (1 × 4 cm, Fig. 1F, step iv), then
217 dried by vacuum freeze-drying. The paper strip biosensor was immersed in LB broth with
218 samples at 37 °C for 1.5 h (Fig. 1F, step v). Once removed, the color development reagent
219 (X-gal) was added to the biosensor cells (Fig. 1F, step vi) before imaging and post-image
220 processing (Fig. 1F, step vii).

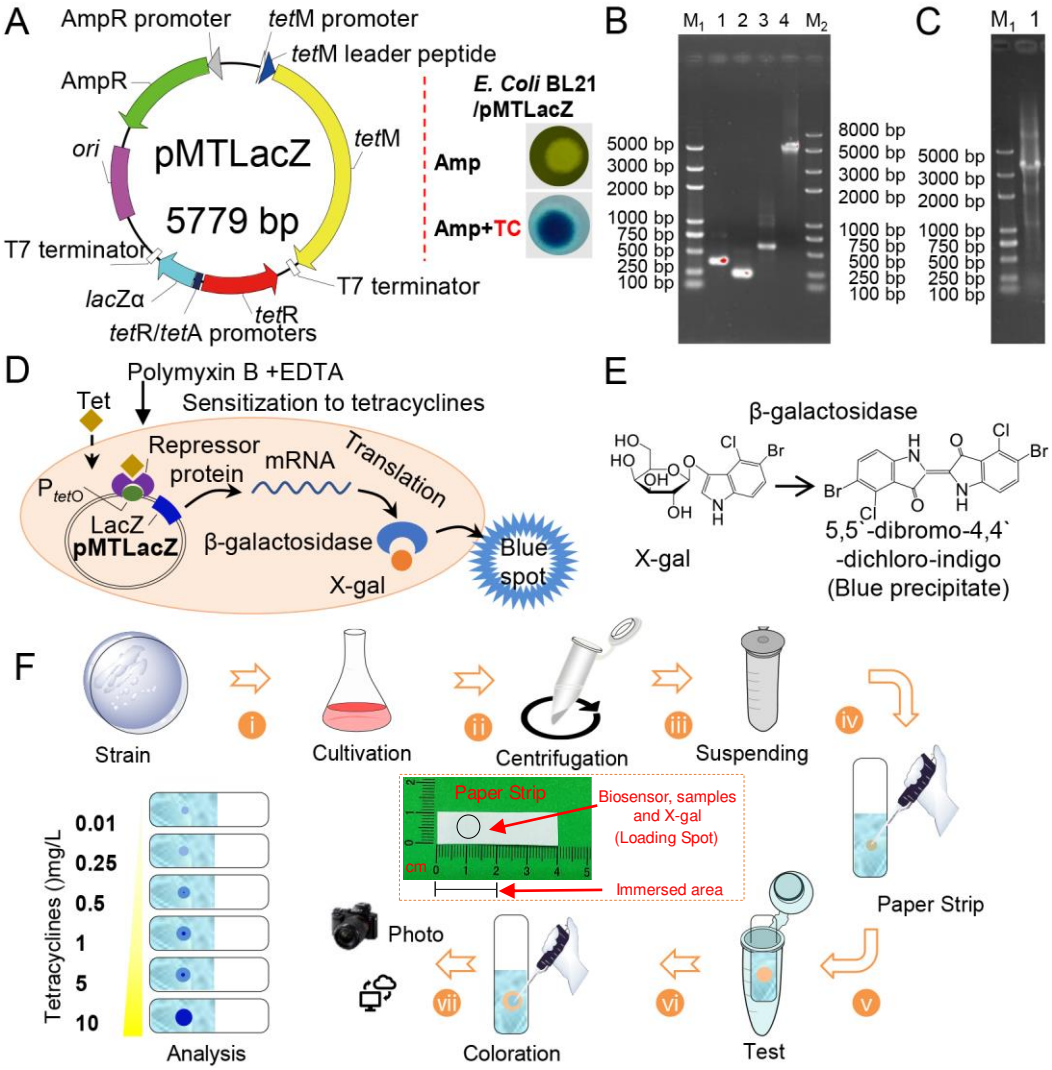

Fig. 1 The construction of the recombinant plasmids. Schematic of the recombinant plasmid (pMTLacZ) and the fluorescence response of the bacteria (*E. coli* BL21/pMTLacZ) induced by 20 mg/L tetracycline (TC) (**A**), Agarose gel electrophoresis of fused gene fragment amplified by PCR (M_1-DNA marker DL5000, 1-*lacZ*, 2-T7, 3-*lacZ*-T7, 4-pMT, M_2-*Trans*2K® Plus II DNA Marker) (**B**), Agarose gel electrophoresis of cloning gene verified by PCR (M-DNA marker DL5000, 1-pMTLacZ partial fragment) (**C**). Genetic organization and mechanism of tetracycline-regulated TC-resistance determinant (**D**). The coupled enzyme reactions were catalyzed by β-galactosidase producing the blue signal (**E**). Tetracycline semi-quantification processing by whole-cell paper strip biosensor (F). Biosensor cultivation (i), preparation of paper strip biosensor (ii–iv), Semi-quantitative analysis of samples using biosensor (v–vii).

3.2 Matrix matched calibration of paper strip biosensors for water and soil extracts

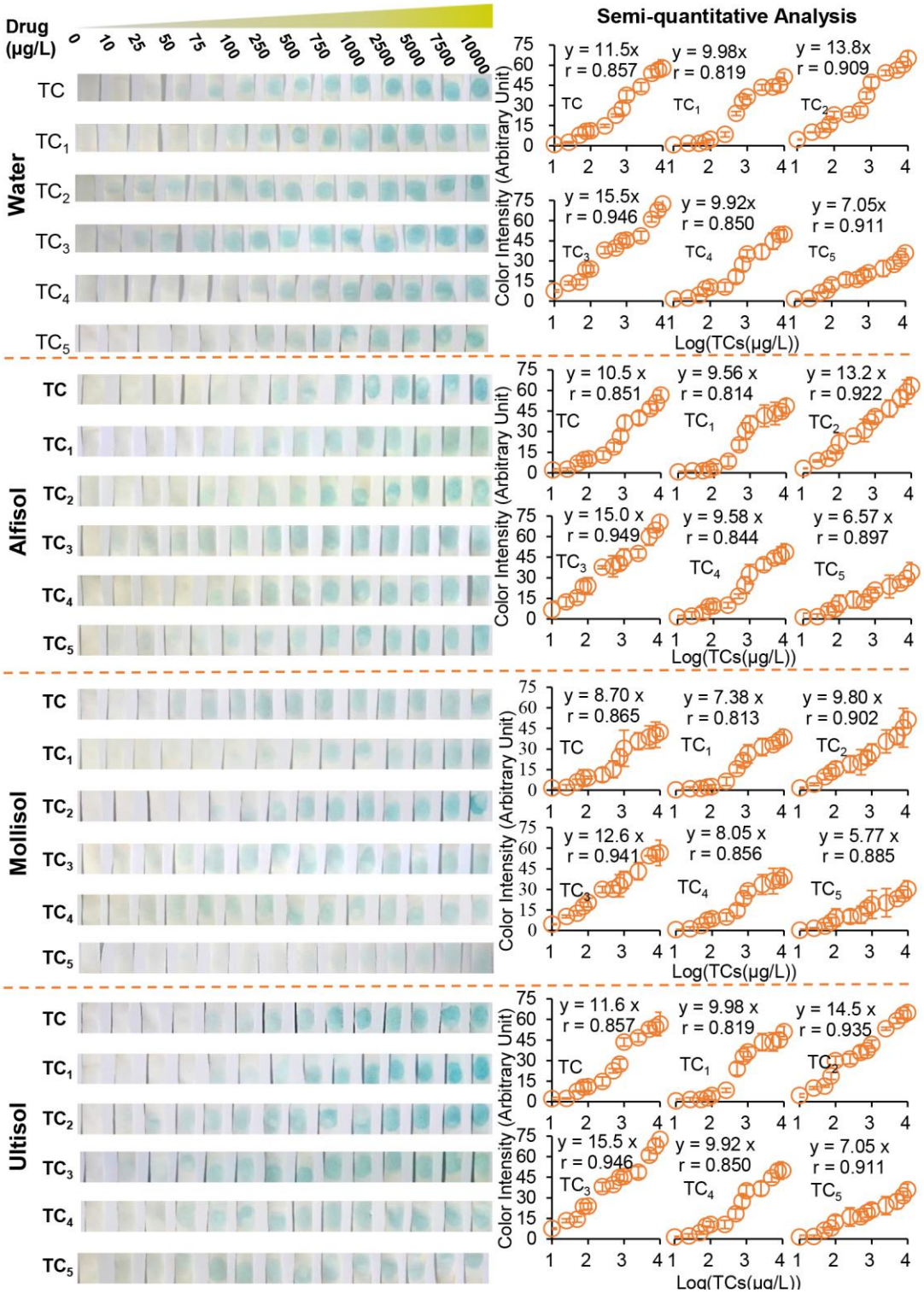

Fig. 2 Matrix matched calibration of paper strip biosensors for water and soil extracts and the correlations between spiked tetracycline concentrations in and color intensity measured using the software ImageJ upon digital image acquisition.TC, TC_1, TC_2, TC_3, TC_4 and TC_5 denote tetracycline, oxytetracycline, chlorotetracycline, deoxytetracycline, minocycline, and methacycline, respectively.

For semi-quantification of tetracycline concentrations in the samples, a 14-point

calibration was prepared for each of the six tetracycline compounds (tetracycline, oxytetracycline, chlorotetracycline, deoxytetracycline, minocycline, and methacycline) spiked in aquafarm water at concentrations ranging from 0−10,000 µg/L. An increase in blue color intensity was observed with increasing concentrations of tetracyclines (Fig. 2). The visual results of the paper strip biosensor were able to indicate low tetracycline concentrations down to 10 µg/L upon color development for 90 min. A good linear relationship between the color intensity and log value of tetracycline concentration in water was observed (Fig. 2, $r > 0.850$, $P < 0.01$). The concentration of each tetracycline in water as a function of the color intensity on the paper strip can be expressed as equation 1 and 2 (Table S5, Detailed illustration in SI).

$$\log TC = \alpha I \quad (1)$$

or

$$TC = 10^{\alpha I} \quad (2)$$

where TC is the concentration of tetracycline, I is the color intensity detected by the paper strip biosensors and, and α is the slope of standard curve (Values given in Fig. 2). Using the color intensity reading of the biosensors paper strip and Eq. (2), *TC* can be then calculated by I and α (Fig. 2) obtained from their corresponding standard calibration curve. After 90 min incubation, good linearity in the range of 75–10000 µg/L were found by paper strip biosensors for six tetracyclines (Table 1). The detection limits of the biosensor method in water were between 5.23 and 17.1 µg/L for all types of soils and tetracyclines as determined by S/N ratio. The paper strip biosensor produced a slightly lower detection limit for chlorotetracycline (5.44 µg/L) and deoxytetracycline (5.23 µg/L) than for other

tetracyclines in water. In comparison, Zhu et al. (2010) obtained a detection limit of 0.20−0.28 μg/L for tetracyclines in groundwater by HPLC. Although the primary aim of this study was to develop a paper strip biosensor for the onsite, high-throughput screening of tetracyclines in water, it is conceivable that the detection limits achieved by HPLC could also be achieved by the biosensor if concentration of the water samples was conducted (Zhu et al. 2001).

Table 1 Detection parameters of six tetracyclines measured by biosensors paper strip in water and soil extracts (Alfisol, Mollisol, and Ultisol)

TCs	Water		Alfisol		Mollisol		Ultisol	
	DL (μg/L)	LR (μg/L)	DL (μg/L)	LR (μg/L)	DL (μg/L)	LR (μg/L)	DL (μg/L)	LR (μg/L)
TC	5.86	25-10000	5.88	25-5000	20.4	50-7500	5.32	50-10000
TC_1	5.85	75-10000	5.95	75-10000	30.1	75-10000	5.76	75-7500
TC_2	5.44	25-10000	5.78	25-7500	19.1	10-10000	5.68	25-10000
TC_3	5.23	10-10000	5.67	25-10000	17.0	10-10000	5.21	25-10000
TC_4	17.1	75-10000	23.4	25-10000	30.2	25-7500	15.4	50-7500
TC_5	9.05	75-10000	35.3	10-10000	28.4	25-7500	12.6	10-7500

TCs, TC, TC_1, TC_2, TC_3, TC_4 and TC_5 are tetracyclines, tetracycline, oxytetracycline, chlorotetracycline, deoxytetracycline, minocycline, and methacycline, respectively. DL and LR are detection limit and Linear range, respectively.

The results above demonstrated the capacity of paper strip biosensors for semi-quantitative detection of tetracyclines in water samples. Extractable fractions of tetracyclines in soils are often used to assess their bioavailability to biota and their mobility through soil profiles (Ikehata et al. 2006; Li et al. 2016; Liu et al. 2014). The EDTA extractable fraction of tetracyclines can be used to evaluate their corresponding environmental and health risks in soils (Bergan et al. 1973; Cipullo et al. 2018; Hansen and Sørensen 2001). Therefore, further validation of the dose-response curves of six tetracyclines from soil extracts using the paper strip biosensor was carried out using a matrix matched approach of spiked soil extracts from three different soils. The sensitivity of tetracyclines in soil extracts, as measured by the color intensity of the paper strip

biosensor response decreased from Ultisol and Alfisol to Mollisol (Fig. 2). The visual detection limit of the paper strip in soil extracts was between 25 and 100 µg/L for all of the soils and tetracycline compounds. Measurement of the color intensities using appropriate software were shown to enhance sensitivity (Fig. 2). Image analysis indicated that the logarithmic concentration of each tetracycline in the soil extracts as a function of the color intensity followed also followed equation 1 ($r > 0.813$, $P < 0.01$) (Fig. 2). A good linearity in the range of 75–7500 µg/L was determined for the paper strip biosensors for all six of the tetracyclines in the soil extracts (Table 1). The detection limits of tetracyclines in the EDTA-extracts of Inceptisol, Mollisol, and Ultisol were 5.67−35.3, 17.0−30.2, and 5.21−15.4 µg/L for this biosensor method (Table 1). Similar to the water, chlorotetracycline and deoxytetracycline showed the lowest paper strip biosensor detection limit in all three soil extracts whereas minocycline and methacycline were highest at 5.21 µg/L in all three soil extracts. Overall, a lower limit of detection was observed in EDTA-extracts from Ultisol than either Inceptisol or Mollisol. The detection limits of the EDTA-extractable tetracyclines in three soils was calculated as $DL = (DL_{SE} \times 1\ mL)/1\ g$ (DL_{SE} denote the detection limit of tetracyclines in soil EDTA-extracts). So, the detection limit of the biosensor method for EDTA-extractable tetracyclines in three soil follow the same principles (5.21–30.2 µg/kg, TableS7).

3.3 Analysis of tetracyclines in contaminated water samples

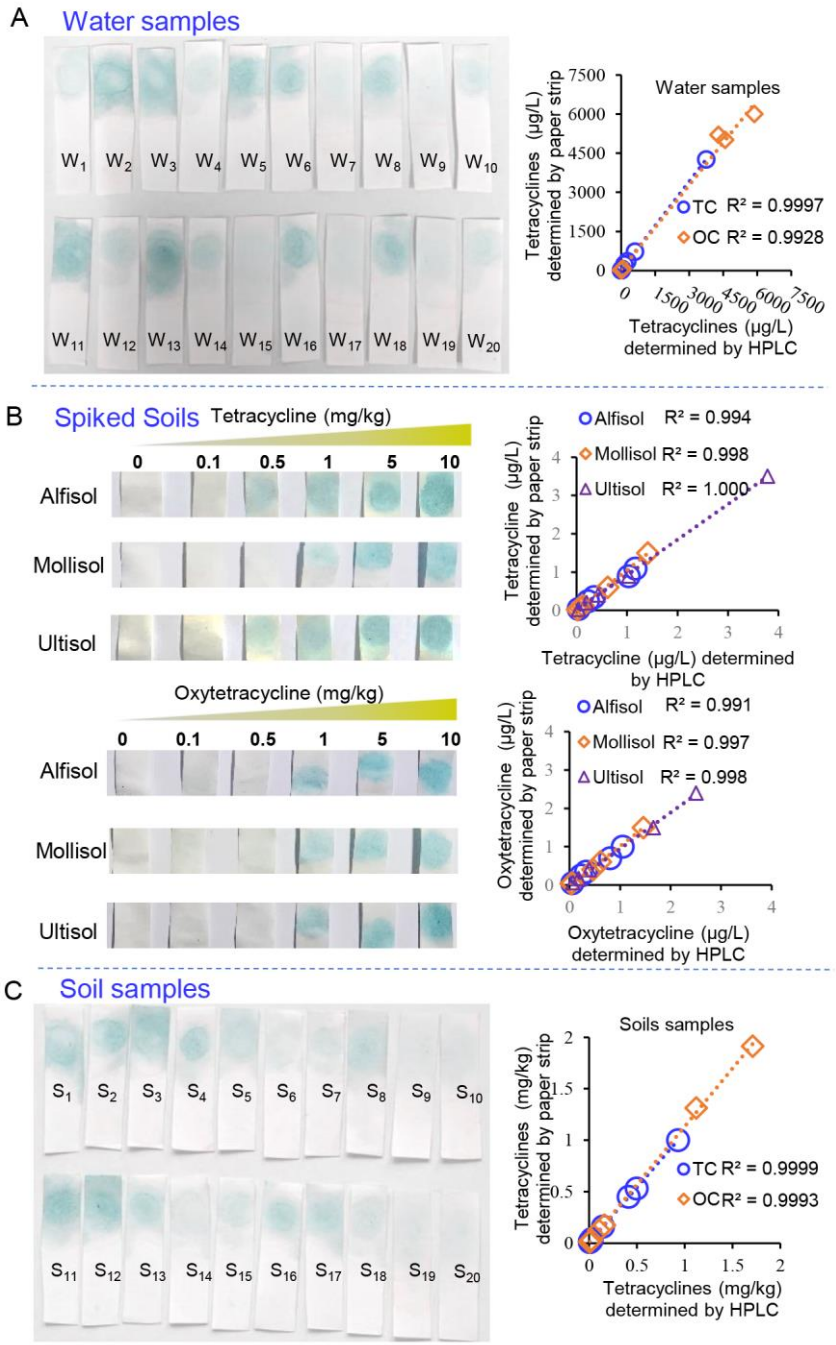

Fig. 3 Tetracycline (TC) and oxytetracycline (OC) semi-quantitation in twenty water samples (A) and twenty soil samples (B) and tetracyclines-spiked soils (C) by whole-cell paper strip biosensors. Results represent the average of triplicate assays whereby prepared whole cell paper strip biosensors were incubated in soil extracts at 37 °C and color intensity measured using the software ImageJ upon digital image acquisition.

The utility of the paper strip biosensor was verified with the analysis of real water

317 samples. For the samples collected from fishponds, the data (Fig. 3A) were almost
318 identical to those obtained from water samples in laboratory, which indicates the sample
319 matrices had a negligible effect on the sensitivity of the biosensors for tetracyclines. For
320 twenty water samples collected from local fishponds in Nanjing (China), the paper strip
321 biosensor indicated that tetracycline concentrations of W_1−W_{10} were 45.8, 3756, 609, 6.32,
322 263, 144, 7.75, 15.4, 7.9 and 6.78 μg/L, respectively (Fig. 3A and Table S7). The
323 tetracycline concentrations of W_1-W_{10} water samples measured by HPLC were 59.7, 4260,
324 718, 9.26, 350, 230, 12.2, 24.3, 11.2 and 10.4 μg/L, respectively, and were slightly higher
325 than the results generated by biosensor method. Standard deviations (between estimated
326 tetracycline concentration by biosensors paper strip and that detected by HPLC, SD) in the
327 analysis of the 10 samples were between 13.4−59.6%. Oxytetracycline concentrations of
328 W_{11}−W_{20} water samples were 4288, 22.4, 5882, 10.7, 66.8, 4585, 13.7, 26.8, 10.8 and 15.2
329 μg/L, respectively (see Fig. 3A and Table S7). The oxytetracycline concentrations of
330 W_{11}−W_{20} water samples measured by HPLC were 5201, 30.2, 6000, 14.2, 78.3, 5001, 15.2,
331 30.3, 11.3 and 17.0 μg/L, respectively, and again were higher than the detected values by
332 paper strip. The SD in the analysis of 10 samples contained oxytetracycline were
333 2.01−33.5% (Fig. 3A and Table 2).
334
335
336
337 **Table 2** Tetracycline (TC) and oxytetracycline (OC) semi-quantitative values in twenty water samples and
338 twenty soil samples by whole-cell paper strip biosensors

Sample	CI	PC (mg/kg)	HC (mg/kg)	SD (%)
W_1	19.1	45.8	59.7	30.2
W_2	41.1	3756	4260	13.4
W_3	32.0	609	718	17.8
W_4	9.21	6.32	9.26	46.5
W_5	27.8	263	350	32.9
W_6	24.8	144	230	59.6
W_7	10.2	7.75	12.2	57.8
W_8	13.7	15.4	24.3	57.7
W_9	10.3	7.90	11.2	42.4
W_{10}	9.56	6.78	10.4	52.6
W_{11}	36.3	4288	5201	21.3
W_{12}	13.5	22.4	30.2	34.9
W_{13}	37.6	5882	6000	2.01
W_{14}	10.3	10.7	14.2	33.5
W_{15}	18.2	66.8	78.3	17.2
W_{16}	36.5	4585	5001	9.09
W_{17}	11.3	13.7	15.2	11.4
W_{18}	14.3	26.8	30.3	13.3
W_{19}	10.3	10.6	11.3	5.71
W_{20}	11.8	15.2	17.0	12.0
S_1	28.3	0.498	0.532	6.86
S_2	27.5	0.414	0.450	8.72
S_3	31.2	0.928	0.100	7.78
S_4	22.7	0.143	0.161	12.4
S_5	15.3	0.029	0.041	42.5
S_6	13.7	0.020	0.030	48.3
S_7	10.3	0.009	0.016	72.8
S_8	14.2	0.022	0.024	6.22
S_9	9.5	0.008	0.011	41.3
S_{10}	8.6	0.006	0.011	58.1
S_{11}	30.9	1.71	1.91	11.7
S_{12}	29.2	1.12	1.31	17.2
S_{13}	13.2	0.024	0.030	25.4
S_{14}	9.21	0.009	0.010	11.4
S_{15}	9.79	0.011	0.013	25.3
S_{16}	19.3	0.011	0.013	19.0
S_{17}	21.1	0.163	0.172	5.90
S_{18}	13.0	0.023	0.030	33.7
S_{19}	7.26	0.006	0.008	43.4
S_{20}	8.32	0.007	0.011	40.9

Color Intensity (CI, Arbitrary Unit), tetracycline concentration measured by paper strip biosensors (PC), concentration determined by HPLC (HC), and standard deviation (SD) between estimated tetracycline concentration, PC, and HPLC determined tetracycline concentration (HC) are presented.

When the values calculated by the biosensor were plotted against the HPLC derived concentrations, a strong linear relationship was established. (R^2 > 0.990, Fig. 3A). This shows that variation between results obtained from the two methods are not concentration dependent and suggests the potential to correct for the underestimated concentrations observed via biosensor analysis.

3.4 Analysis of extractable tetracyclines fraction in contaminated soil

The paper strip biosensor was also validated for the analysis of the extractable tetracycline fraction in contaminated soils following the same extraction procedure from soil as described above. The results of the analysis in tetracycline and oxytetracycline-spiked soils revealed that the concentrations of extractable tetracycline measured by paper strip biosensors were closer to the values of extractable tetracycline concentration measured by HPLC (Fig. 3B). Two tetracyclines concentrations by whole-cell paper strip biosensors showed a good linear relationship with the concentrations of that of detection by HPLC (R^2 > 0.991). The paper strip was further tested to detect tetracycline concentrations in soils sampled from a test field that were contaminated with either tetracycline or oxytetracycline. Using the paper strips, the extractable tetracycline concentration in S_1-S_{10} soils ranged from 0.006−0.498 mg/kg, (Fig. 3C and Table 2), which were slightly lower than the concentrations measured by the HPLC method (0.011−0.532 mg/kg). The extractable oxytetracycline concentrations in S_{11}-S_{20} soils measured by paper strip biosensors ranged from were 0.006−1.71 mg/kg which were very similar to the concentrations measured by HPLC (0.008−1.91 mg/kg). The SD values of the extractable tetracycline in soils measured by the paper strip

biosensor S_1-S_{10} (6.86−72.8%) were greater than the SD values of the extractable oxytetracycline in S_{11}-S_{20} soils (5.90−43.4%). However, both SDs provide adequate reproducibility for a semi-quantitative method. The same as for water, a strong linear relationship existed between the concentrations of both compounds determined by paper strip biosensors and HPLC (R^2 > 0.999, Fig. 3C and Table 2). Combined with no observable matrix interferences, these results confirm that these paper strip biosensors provide reliable semi-quantitative evaluation of tetracycline concentrations from soil extracts.

3.5 Advantages

The paper strip biosensors include greater ability to accommodate the variation of environmental conditions in water or soil, such as a wide range of ionic strengths, pH (4−8), and temperature (Iglesias et al. 2009) that is hard to overcome the impact other types of paper strip sensors such as those based on immunochromatographic lateral flow and immobilization of antibodies. This biosensor is more robust and has an excellent capacity to endure various environmental conditions, such as those expected in pond water and agricultural soil where contamination with tetracycline antibiotics is commonly associated with the utilization of feed additives in aquaculture and the use of livestock manure as a soil fertilizer amendment.

In this study, the construction of *Escherichia coli*/pMTLacZ for whole-cell biosensor was similar to that of *Escherichia coli*/pMTGFP and *Escherichia coli*/pMTmcherry from our previous study (Ma et al. 2020). These cellular reporters can rapidly and accurately detect tetracyclines in water or soil. However, cost effective and readily available Whatman filter paper was utilized as the carrier for the biosensor scaffolding (Fig. 1), which has substantially greater portability and is more economical than the 96-microwell plate method described in previous studies (Wang et al. 2010; Zou et al. 2018). Biosensor

paper strips were produced by a simple culture process (3 hours at 37 °C with vigorous shaking at 150 r/min) in cheap media (Lysogeny Broth), which dramatically reduces analysis cost. This semi-quantitative method has adequate sensitivity and high selectivity, only detecting tetracycline antibiotics, and requires minimal sample pretreatment. This paper strip biosensor offers a lower analysis cost and little instrumental expertise compared to conventional analytical methods (gas chromatography, liquid chromatography, mass spectrometry) (Aga et al. 2016; Arefev et al. 1987). Meanwhile, developing EDTA solvent extraction for the detection of tetracyclines in soils is an inexpensive, easily accessible and environmentally-friendly method compared to many common methods which use organic solvents needed to perform chromatographic separations (Batt and Aga 2005; Hamscher et al. 2005). The combination of EDTA and polymyxin B as the agent to sensitize this material resulted in significantly improve method precision and accuracy (Belkin 2003; Parlanti et al. 2000).

At present, portable colorimeters and office scanners pixelated by GIMP software into an RGB profile can evaluate the color intensity of the paper strip, but it's exorbitant for onsite application and not nearly as portable as reagent kits. In our study, optical changes of the sensor were acquired by a digital camera and analyzed by Image J software that enhanced the portability of these paper strip kits while vastly reducing the expense of antibiotic detection. Based on the image processing described in this study, the color analysis software could be developed as a mobile phone APP to directly capture and analyze images and output detection results which would increase user-friendliness while further decreasing analysis time.

4. Conclusion

We have developed a self-contained portable sensing system based on genetically engineered whole-cells physically adsorbed on filter-paper strips for on-site

semiquantitative visual monitoring of tetracyclines in environmental samples. Additionally, we demonstrate their ability to provide quantitative measurements by means of digital image analysis. This novel filter-paper strip biosensor method obtained a quantification range of 75–10000 μg/L with detection limits of 5.23–17.1 μg/L for 6 tetracycline antibiotics in water and a detection limit of 5.21–35.3 μg/kg for the EDTA-extractable tetracyclines from three soils. This study describes a fast and convenient method for the detection of tetracyclines, which could be employed for first-level screening of a variety of environmentally and clinically relevant samples. A filter-paper-based biosensor provides easy transportation and storage, and does not require instrumentation or trained personnel; therefore, it could be a component of a simple and inexpensive field kit.

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Competing interests

The authors declare no competing interests.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Additional information

Supplementary information is available for this paper at

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