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Rapid Detection of Tetracyclines and Their 4-Epimer Derivatives from Poultry Meat with Bioluminescent Biosensor Bacteria

NINA E. VIROLAINEN,*,† MARIËL G. PIKKEMAAT,‡ J. W. ALEXANDER ELFERINK,‡ AND MATTI T. KARP†,§

Department of Chemistry and Bioengineering, Tampere University of Technology, P.O. Box 541, 33101 Tampere, Finland, RIKILT—Institute of Food Safety, Wageningen University and Research Centre, P.O. Box 230, 6700 AE Wageningen, The Netherlands, and BCC—Biosensing Competence Centre, P.O. Box 692, 33101 Tampere, Finland

Tetracycline (TC) specific luminescent bacterial biosensors were used in a rapid TC residue assay sensitized to meet the EU maximum residue limit (MRL) for TC residues in poultry muscle tissue (100 μg kg^{-1}) by membrane-permeabilizing and chelating agents polymyxin B and EDTA. Sensitivities of 5 ng g^{-1} for doxycycline, 7.5 ng g^{-1} for chlortetracycline, and 25 ng g^{-1} for tetracycline and oxytetracycline were reached. Except for doxycycline, the MRLs of these tetracyclines include their 4-epimer metabolites. In the biosensor assay, all four 4-epimers showed induction capacity and antimicrobial activity, and antimicrobial activity was also observed in the inhibition assay, although with lower efficiency than that of the corresponding parent compound in both assays. The biosensor assay is an inexpensive and rapid high-throughput screening method for the detection of 4-epimer TC residues along with their parent compounds.

KEYWORDS: Tetracycline; whole-cell biosensor; bacterial luciferase operon; polymyxin B; food samples; antibiotic residue; tetracycline metabolites

INTRODUCTION

Antibiotics are used in animal husbandry for therapy and disease prevention and, outside the EU, for growth promotion. Tetracyclines are among the most widely used veterinary antibiotics: in 1997, 66% (2294 t) of the total sales of veterinary antibiotics over the EU and Switzerland consisted of tetracyclines (*1*), whereas in The Netherlands, tetracyclines comprised 58% of sold veterinary antibiotics in 2006 (*2*). Antibiotic residues in food products may lead to selection of antibiotic-resistant bacteria in the human gut after the food is consumed (*3*). Allergic reactions, potential toxicity to humans, and inhibition of starter cultures in the production of fermented milk products are other reasons for controlling antibiotic usage and testing for residues in food products (*4*).

EU legislation enforces countries to establish and execute a national monitoring plan, under which a set percentage of animal products should be monitored for antibiotic residues and other contaminants (*5*). An EU community procedure for the establishment of maximum residue limits (MRLs) of veterinary medicinal products in foodstuffs of animal origin became effective in 1990 (*6*). The 1999 amendment consolidated the MRLs of the four veterinary tetracyclines tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC), and doxycycline (DC) at 100 μg kg^{-1} in muscle, other matrixes receiving similar or higher MRLs (*7*).

Screening for antimicrobial residues is preferably done by immunological or microbial methods because of their high cost effectiveness. Immunological tests are fast and specific, but relatively expensive, whereas microbiological tube tests are relatively fast (3−4 h) and easy to perform, but have difficulty detecting tetracycline residues at their MRL (*8*). Effective group-specific detection of tetracyclines can be achieved by implementing a plate assay containing *Bacillus cereus* ATCC 11778 as the test organism (*9, 10*). However, the disadvantage of this type of assay is that it is rather voluminous and requires overnight incubation. Consequently, there is a need for fast and high-throughput screening methods which are affordable and simple to perform. Antibiotic group-specific whole-cell biosensor strains might answer this need.

This work employed the bioluminescent whole-cell biosensor strain TetLux developed for detection of tetracyclines (*11*). These *Escherichia coli* biosensor cells contain a plasmid featuring the *Photorhabdus luminescens*-derived bacterial luciferase operon placed under control of the tetracycline-responsive elements of transposon Tn*10*. The regulation system involves repressor protein TetR, whose affinity to the operator

* To whom correspondence should be addressed. E-mail: nina.virolainen@tut.fi. Phone: 358 3 3115 2570. Fax: 358 3 3115 2869.
† Tampere University of Technology.
‡ Wageningen University and Research Centre.
§ BCC—Biosensing Competence Centre.

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sequence in p_{tetA} is reduced by TC binding, thus allowing transcription from the promoter. The use of the bacterial luciferase operon confers the strain a self-luminescent phenotype, i.e., bioluminescence production without the addition of exogenous substrates. This feature makes the use of the cells as sensor elements straightforward particularly when they are used in a freeze-dried format, which facilitates reagent-like use of biosensor cells in the assay. We have used TetLux biosensors in developing a rapid assay for analyzing the presence of residues of tetracyclines in a new sample matrix, poultry meat, and studied the effects of tetracycline metabolites in the assay. Assay sensitization was achieved by a new method, the combined use of two membrane-permeabilizing agents, one of which also exerts divalent cation chelating activity.

MATERIALS AND METHODS

Reagents Used in the Study. $NaH_2PO_4 \cdot 2H_2O$, $Na_2HPO_4 \cdot 7H_2O$, NaCl, and EDTA disodium salt dihydrate were from Merck KGaA, Darmstadt, Germany. Tryptone and yeast extract were from Difco Laboratories, Le Pont de Claix, France. Ampicillin, tetracycline hydrochloride, doxycycline hydrochloride, chlortetracycline hydrochloride, oxytetracycline hydrochloride, and polymyxin B sulfate were from Sigma, St. Louis, MO. 4-Epitetracycline hydrochloride, 4-epichlortetracycline hydrochloride, and 4-epioxytetracycline hydrochloride were from Acros Organics, Geel, Belgium. 4-Epidoxycycline was a kind gift from Prof. K. Eger, Institute of Pharmacy, University of Leipzig, Germany. Tetracycline stock solutions were prepared by dissolving tetracycline hydrochloride powders initially in 0.1 M HCl (5%, v/v, of the final volume) and then in 0.1 M phosphate buffer, pH 6.0 (95%, v/v, of the final volume). The 4-epimer stock solutions were prepared in demineralized H_2O. Stock solutions were stored at 4 °C and renewed every four weeks. Fresh dilutions from stocks were used in all experiments.

Bacterial Strains, Plasmids, and Media. The growth inhibition assay was performed in Iso-sensitest agar from Oxoid, Basingstoke, U.K., using *B. cereus* ATCC 11778 as the indicator organism. The construction of the tetracycline biosensor strain *E. coli* K12(pTetLux1) is described in ref *11*. In short, the *P. luminescens* bacterial luciferase operon *luxCDABE* obtained from pCGLS-11 was inserted as an *Eco*RI fragment under control of the tetracycline-inducible *tetA* promoter/operator in pASK75. The resulting plasmid construct, named pTetLux1, was transformed by electroporation into *E. coli* K12 strain M72 to obtain a bioluminescent tetracycline-responsive bacterial biosensor strain. The sensor bacteria were cultured at 37 °C with aeration (200 rpm) in Luria−Bertani broth (LB; 1% (w/v) tryptone, 0.5% (w/v) yeast extract, 0.5% (w/v) NaCl, pH 7.0) supplemented with 100 μg mL^{-1} ampicillin (LBAmp).

Lyophilization of the Tetracycline Biosensors. The biosensor cells were lyophilized to ensure reagent-like behavior in the assay. Tetracycline sensor bacteria were cultivated in LBAmp medium to $A_{600\,nm} = 1.5$ as described above. The cells were harvested by centrifugation (1500*g*, 15 min) and suspended in fresh LB supplemented with 10% (w/v) lactose using a volume of one-quarter of the original. The cell suspension was divided into 0.5 mL aliquots in glass vials (Schott AG, Mainz, Germany) and lyophilized using a 24 h procedure (*12*). After freeze-drying, the vials were sealed under a nitrogen atmosphere and stored at −20 °C.

Preparation of Tetracycline-Spiked Poultry Meat Fluid Samples. Poultry meat used in the study was organically produced chicken breast fillet obtained from a butcher shop in Wageningen, The Netherlands. The tetracycline stock solution was diluted in demineralized H_2O to achieve the wanted concentration, and 2.5 mL of dilution was added in a 97.5 g portion of chicken meat to obtain a total of 100 g of spiked meat. To obtain a blank sample, pure demineralized H_2O was used for spiking. The spiked meat was minced in a Moulinette blender (Moulinex), incubated at 4 °C for 1 h, and weighed into centrifuge tubes as 25 g portions. The meat was heated in a 64 °C water bath for 20 min and centrifuged for 10 min at 27000*g*. The supernatant (meat fluid) was collected by decanting and stored at −20 °C.

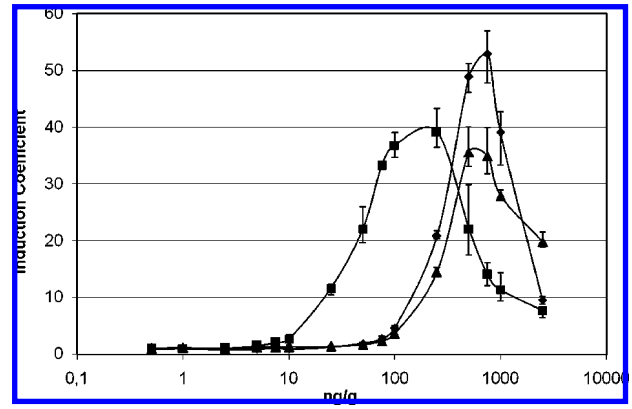

Figure 1. Tetracycline dose−response curves in a plate assay performed using cultured or lyophilized TetLux cells. Lyophilized cells were rehydrated either in the absence or in the presence of 0.5 μg mL^{-1} PMB. Results of three parallel experiments are shown. Key: ■, cells rehydrated with PMB; ◆, cells rehydrated without PMB; ▲, cultured cells.

Tetracycline Bioassays. *Plate Assays.* Tetracycline plate assays were performed using either lyophilized biosensor cells or cells cultured in LBAmp. Lyophilized cells were reconstituted by adding 2 mL of LB per vial to reach the same density the cells had before lyophilization and incubating at room temperature for 1 h. Reconstituted cells were then diluted 1:4 in LB phosphate-buffered (100 mM) to pH 6 (LBPB). The biosensor strain was cultured in LBAmp from a 2% inoculation of an overnight culture to $A_{600\,nm} = 1.5$ as described above. The cultured cells were diluted 1:100 in LBPB. Reconstituted or cultured cells were divided onto white 96-well flat bottom microtiter plates (Greiner Bio-One GmbH, Frickenhausen, Germany) as 50 μL aliquots. Beforehand, the wells had been dispensed with 150 μL of meat fluid sample and 25 μL of 225 mM EDTA solution to obtain a final EDTA concentration of 25 mM. The plates were sealed by tape and incubated for 3 h at 30 °C and 300 rpm. Bioluminescence was read from the plates by a Synergy HT multidetection microplate reader (BioTek Instruments Inc., Winooski, VT).

Assay Sensitization with Polymyxin B. Polymyxin B (PMB) was added to biosensor cells to render them more sensitive to tetracyclines in the plate assay. PMB was added to rehydration medium of lyophilized cells in a concentration of 0.5 μg mL^{-1} and allowed to affect the cells during reconstitution. An equal concentration of PMB was applied to LBPB used to dilute the rehydrated cells prior to distribution of the cells on microtiter plates. The tetracycline bioassay was completed as described above.

Microbiological Inhibition Test. For preparation of the microbiological inhibition test plate, 31.4 g L^{-1} Iso-sensitest agar was sterilized for 15 min at 121 °C. After the agar was cooled to 48 °C, the pH was adjusted to 6.0 ± 0.1 if necessary. Chloramphenicol was added to a final concentration of 0.5 mg L^{-1}. This medium was inoculated with *B. cereus* ATCC 11778 at 10^5 cfu mL^{-1} of agar and immediately poured as a layer of approximately 2.2 mm. After solidification, holes with a diameter of 14 mm were punched into the plate. A sample volume of 250 μL was applied to the sample hole and supplemented with 25 μL of 1 M phosphate buffer, pH 6, containing 20 mg L^{-1} chloramphenicol. After overnight incubation at 30 °C, inhibition diameters were measured using a vernier calliper.

RESULTS

Performance of TetLux Cells in a TC Assay from Poultry Meat Fluid. Tetracycline biosensor strain *E. coli* K12(pTetLux1) was used in this study to develop a rapid detection method for tetracycline traces in poultry meat. The sample material was meat fluid extracted from chicken breast muscle tissue. To facilitate reagent-like behavior, biosensor cells were mainly used in a lyophilized form, but cultured cells were tested in the assay to provide proof of the functionality of lyophilized cells (**Figure 1**). The signals were read from the plate after 3 h of induction,

Table 1. Relative Induction Coefficients from a Plate Assay Performed with TetLux Cells Rehydrated in the Presence of Various PMB Concentrations[a]

[PMB], μg mL^{-1}	50 ng g^{-1} OTC	100 ng g^{-1} OTC
0	1	1
0.1	0.9 ± 0.2	1.1 ± 0.3
0.25	5.4 ± 1.3	5.0 ± 0.4
0.5	19.8 ± 1.5	9.1 ± 0.4
0.75	24.8 ± 7.2	11.0 ± 4.8
1.0	13.6 ± 8.4	5.9 ± 3.8
2.5	5.9 ± 1.6	2.1 ± 0.4

[a] The table presents results from samples spiked to two different OTC concentrations: 50 ng g^{-1} (below the MRL) and 100 ng g^{-1} (MRL). Results of two parallel experiments are shown.

giving the assay a total duration of 4 h. Assay results are reported as induction coefficients (ICs) calculated by dividing the signal by the background, the latter being the nonspecific signal from wells containing blank meat fluid as a sample. The limit of detection was then set as the concentration where IC exceeded the value 3 in all parallel assays (*13*). **Figure 1** shows a similar dose-responsive behavior between cultured and lyophilized cells. A suitable assay should be able to detect residue levels well below the MRL. The limit of detection was exactly 100 ng g^{-1} for TC (**Figure 1**) and above the MRL for OTC (not shown). On the basis of the Mg^{2+} ion concentration dependency of the p_{tetA} induction efficiency (*14*), a method for TetLux assay sensitization involving the use of chelating agents has been reported (*15*). However, in this study the use of the chelating agent EDTA alone did not sensitize the assay to concentrations below the MRL values (results not shown). Consequently, we sought an alternative method for TetLux assay sensitization.

Cooperative Assay Sensitization with Membrane-Permeabilizing and Chelating Agents. *Polymyxin B as a Sensitizing Agent.* PMB is a lipopeptide antibiotic that disrupts the integrity of the Gram-negative outer membrane. The addition of PMB to rehydrating cells in sublethal concentrations produced outstanding results in sensitizing the assay (**Table 1**). The maximal IC was actually lower in the presence of PMB probably due to its effect on cell viability, but the IC from a sample below the MRL (50 ng g^{-1} OTC, the least potent inducer of all four tetracyclines tested) reached 20-fold higher values compared to that of an assay with no PMB present. The highest ICs were obtained using 0.75 μg mL^{-1} PMB, but we opted for 0.5 μg mL^{-1} PMB to be used in further assays since higher PMB concentrations clearly had a detrimental effect on cell viability as seen from a decrease in absolute signal levels. **Figure 1** also presents results from a sensitized TetLux assay using cells reconstituted with and without PMB. The limit of detection improved from 100 to 25 ng g^{-1} for TC, making it feasible to detect tetracycline residues present in concentrations below the MRL.

EDTA as a Sensitizing Agent. To see whether the addition of chelating agents would further increase assay sensitivity, we tested different EDTA concentrations in the assay using TetLux cells lyophilized with 0.5 μg mL^{-1} PMB. In contrast to TC standard solution samples, where the addition of EDTA lowered the absolute signals and ICs, meat fluid spikes required the presence of EDTA to produce high absolute signals and ICs (**Table 2**) due to a higher Mg^{2+} concentration that apparently hinders the entry of tetracyclines into the cells. Of the four EDTA concentrations tested, a final concentration of 35 mM EDTA in the well produced the highest ICs. However, 25 mM was chosen to be used in the assays since the absolute signals were lowered by the higher EDTA concentration even if the ICs were higher. Like PMB, EDTA increases the permeability of the outer membrane and therefore has a detrimental effect on cells in high concentrations (*16*). Media buffered to pH 6 was used in the assays to prevent the alkaline EDTA solution from elevating the pH in the wells. This improves TC entry into the cells since TCs accumulate in the compartment with higher pH (*14*).

Table 2. Relative Induction Coefficients from a TetLux Plate Assay Performed with Various EDTA Concentrations[a]

[EDTA], mM	50 ng g^{-1} OTC	100 ng g^{-1} OTC
0	1	1
5	1.8 ± 0.8	2.5 ± 0.3
10	8.2 ± 6.0	9.6 ± 2.2
25	23.2 ± 7.4	16.2 ± 3.6
35	31.3 ± 15.8	20.0 ± 0.1

[a] Samples were spiked to two different OTC concentrations: 50 ng g^{-1} (below the MRL) and 100 ng g^{-1} (MRL). Results of two parallel experiments are shown.

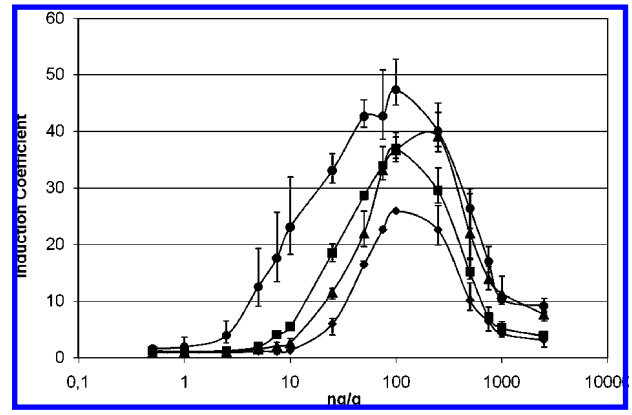

Figure 2. Dose−response curves for four veterinary tetracyclines in a plate assay sensitized with PMB and EDTA. Results of three parallel experiments are shown. Key: ●, DC; ■, CTC; ▲, TC; ◆, OTC.

Performance of the Sensitized TetLux Assay. Dose−response curves were determined for four different tetracyclines using lyophilized cells rehydrated in the presence of 0.5 μg mL^{-1} PMB and applying 25 mM EDTA in the assay wells (**Figure 2**). Again, the limit of detection was calculated as the concentration where IC exceeded the value 3 in all parallel assays. The limit of detection was 5 ng g^{-1} for DC, 7.5 ng g^{-1} for CTC, and 25 ng g^{-1} for both TC and OTC. DC and CTC were clearly the most potent inducers as the induction coefficients indicated considerably lower assay sensitivity for the other two antibiotics, OTC and TC.

Tetracycline 4-Epimer Metabolite Detection Capability of the TetLux Assay. Except for that of doxycycline, the MRLs for tetracyclines include their 4-epimer metabolite (*7*). However, 4-epiDC may as well be relevant since it is reported to occur in both turkey liver and muscle (*17*). These facts prompted us to investigate the effect of the 4-epimer metabolites in the TetLux assay. Meat fluid obtained from poultry spiked with the 4-epimer metabolites was observed to harbor induction capacity, but all 4-epimer samples were clearly less efficient inducers than meat fluids spiked with the parent compounds (**Figure 3**). The 4-epimer standards contained 0.1−3% impurity (4-epiTC, 4-epiOTC, and 4-epiTC 97% pure and 4-epiDC 99.9% pure), which might consist of the parent compound and thus be responsible for the observed induction. The results, however, indicate a much larger inducing effect than can be attributed to traces of the parent compound. The induction efficiency increased in the following order: 4-epiOTC < 4-epiDC <

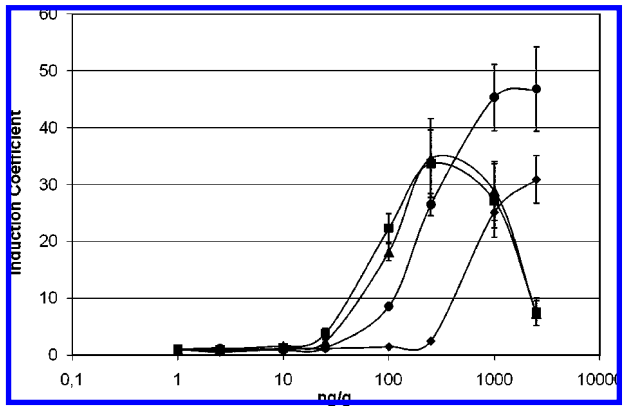

Figure 3. Dose−response curves for the 4-epimers of the four veterinary tetracyclines in a TetLux plate assay. Results of two parallel experiments are shown. Key: ●, 4-epiDC; ■, 4-epiCTC; ▲, 4-epiTC; ◆, 4-epiOTC.

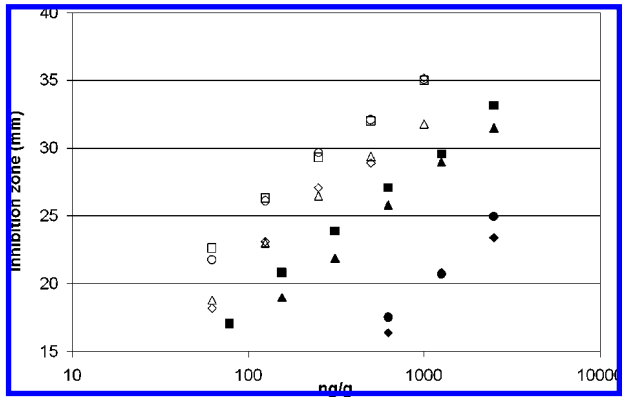

Figure 4. Calibration curves for four veterinary tetracyclines and their 4-epimers in a microbiological inhibition plate assay. Key: ●, DC; ■, CTC; ▲, TC; ◆, OTC; open symbols, parent compounds; closed symbols, 4-epimers.

4-epiTC < 4-epiCTC. This order was slightly different from the induction efficiency order of the parent compounds, OTC < TC < CTC < DC.

Effect of 4-Epimers in a Microbiological Growth Inhibition Assay. The 4-epimer metabolites, particularly 4-epiDC, are generally believed to lack antimicrobial activity. However, 4-epiTC is known to be able to both induce transcription from p_{tetA} (*18*) and exhibit antimicrobial activity (*19*). The results from the induction experiments prompted us to test the 4-epimer metabolites along with the parent compounds in a conventional *B. cereus* microbiological inhibition plate assay. Meat fluid from poultry spiked with the 4-epimer metabolites did yield growth inhibition (**Figure 4**) although at a lower efficiency than meat fluid spiked with the parent compounds. Again, the results obtained in the assay show a higher concentration of inducing compound than the 0.1−3% impurity, indicating that the parent possibly present as an impurity is not the sole antimicrobially active agent. The antimicrobial potency of the parent compounds follows the order TC = OTC < CTC = DC, whereas the 4-epimer potency increases in the order 4-epiOTC < 4-epiDC < 4-epiTC < 4-epiCTC. The order of antimicrobial efficiency of the tetracycline analogues is similar to the order observed in assaying the induction capacity. Therefore, it can be concluded that epimerization at C-4 influences the affinity of all four tetracyclines to both TetR and the 30S ribosomal subunit in a similar manner. The results in **Figures 2−4** show that the biosensor assay is at least as sensitive as the inhibition assay used in routine monitoring of poultry meat and therefore a suitable replacement for the conventional method.

DISCUSSION

The tetracycline biosensor strain *E. coli* K12(pTetLux1) was used in this study to develop a rapid detection method for tetracycline traces in poultry meat. Previously, the strain has been shown to exhibit a dose-responsive behavior with increasing tetracycline concentrations and utilized in detecting tetracycline residues in cow milk (*15, 20*), porcine serum (*21*), and fish tissue (*13*). Except for porcine serum for which no MRL has been set, the results obtained in these studies all showed tetracycline residue detection below the EU MRL set for the sample matrix in question. We optimized the TetLux assay for a new sample matrix, meat fluid extracted from chicken muscle tissue. Only the four veterinarily relevant tetracycline antibiotics were used in this study, but the functionality of the assay with other members of the TC group such as methacycline, demeclocycline, and minocycline has previously been shown (*11*). By using PMB to sensitize the assay, we reached detection limits well below the MRL of 100 ng g^{-1} set for TCs in muscle tissue by the EU. PMB is a lipopeptide antibiotic specific for Gram-negative bacteria. It exerts its antimicrobial activity by forming pores in the outer membrane and at lethally high concentrations in the cytosolic membrane of the cell envelope (*16*). PMB and other antimicrobial peptides active on Gram-positive or eukaryotic cells (reviewed in ref *22*) could be utilized in sensitization of numerous other assays where the analyte needs to enter the cell to evoke a response. Such tests include growth inhibition assays as well as methods based on whole-cell biosensors.

The conditions in the TetLux assay were adjusted for optimal performance in the complex interplay of the assay components. The relatively high Mg^{2+} content of meat fluid (the magnesium content in chicken breast muscle is 10^{-4} mol g^{-1} of tissue (*23*)) was balanced by EDTA, the second substance used for TetLux assay sensitization in this study. Mg^{2+} plays a role in TC accumulation in bacterial cells (*14*). In both the cytoplasm and outside the cell, TC exists in equilibrium between a neutral form and a charged Mg^{2+}−tetracycline chelate. The chelate passes the outer membrane through porins, but dissociation in the periplasmic space is required as only the neutral form can diffuse through the cytoplasmic membrane. Chelation can then recur in the cytoplasm. The extent of TC chelation increases with elevations in pH and Mg^{2+} concentration, causing TC to accumulate in the compartment that has a higher pH and Mg^{2+} concentration. Chelation must occur for TC to be able to exert antimicrobial activity or induce tetracycline resistance since only the chelated form can bind ribosomes or TetR (*18, 24*). In our assay, EDTA aids TC accumulation in the cells by lowering the extracellular Mg^{2+} concentration. Both EDTA and PMB increase the permeability of the outer membrane by removing from the lipopolysaccharide layer divalent cations (Mg^{2+}, Ca^{2+}) that stabilize the outer membrane structure (*16*). Through the membrane-destabilizing action of PMB and EDTA, intracellular TC accumulation is further enhanced.

For three out of the four TCs the EU MRL is set as the sum of the parent and the 4-epimer metabolite (*7*). This was based on the fact that epimerization was thought to occur during sample preparation, which therefore should be accounted for in the determination of the total amount of residue present. For DC this process was assumed to be absent (*25*). However, increasing evidence suggests that the 4-epimer metabolites are also formed in vivo, including 4-epiDC (*17, 26*). There are indications that 4-epimers found in incurred samples are degradation products rather than real metabolites, but are anyhow

formed in vivo (*26*). It is therefore of interest to explore the characteristics and effects of these derivatives in screening assays.

We investigated the effect of the 4-epimer metabolites of four veterinary tetracyclines in the TetLux assay. All four epimers seem to harbor an induction capacity as well as antimicrobial activity. The differences in potency of induction between the tetracycline analogues used in this study are likely to be caused by the differences in association with the repressor protein TetR. No significant differences have been detected in TetR binding of TC and OTC, whereas 4-epiTC had a significantly weaker and CTC and DC a somewhat stronger affinity to TetR than TC (*18*). In the same study, a strong reduction in the induction efficiency of 4-epiTC and an increase in the induction efficiency of CTC and DC were observed. We detected similar results in our study. It has been proposed that the stronger the affinity to TetR, the more extensive the changes in the TetR structure that lead to more efficient induction (*27*). Another factor possibly affecting the potency of induction and entry into cells is the efficiency of Mg^{2+} complex formation, but only slight differences in Mg^{2+} binding between TC and its derivatives 4-epiTC, OTC, DC, and CTC have been detected (*18*). We cannot fully exclude the presence of the parent compound in the assay, as the 4-epimers had a reported purity of 97−99.9%. Also, the possibility of 4-epimer reversion back to the parent conformation during sample preparation or storage should be taken into consideration. The results obtained from both the TetLux and growth inhibition assays indicate a higher concentration of active substance than can be expected from the epimer impurity levels. Also, it has been reported that epimer formation is favored at acidic pH, whereas reversion back to the parent occurs at alkaline pH (*28*). The pH values measured for meat fluid samples were slightly acidic, ranging from 6.0 to 6.2.

Using the test developed here for real screening purposes requires further studies with incurred samples. Although this may slightly change the assay parameters determined as the optimum here, we have verified the robustness of the assay by performing additional experiments with nine individual blank chicken samples, showing that in all cases the optimized assay conditions were adequate to detect OTC concentrations below 50 ug kg^{-1}. Adjustments in the test protocol created in this study will also be needed to prevent the occurrence of false-negative and false-positive results. To rule out false-negative assay results, the high dose hook effect observed in the dose−response curve can be overcome by performing a serial dilution of the sample prior to the assay. The exact dilution protocol and diluent composition are subjects for further studies. Dilutions are also a way to combat false-positive results as several results from one sample give a better estimate of the concentration in the semiquantitative assay. Cutoff values can also be assigned although different IC maxima from different tetracyclines may complicate their use. In the end, the method is meant for screening purposes, so its primary task is to evaluate large numbers of samples for potential noncompliant (>MRL) samples. Positive samples should be analyzed further with confirmatory methods.

Efficient screening methods are required for successful control of residues. These tests should be inexpensive, rapid, and easy to perform and should allow processing of a multitude of samples. Our whole-cell biosensor TC detection method has the potential to meet all these qualifications as biosensor cells are inexpensive to produce, the test can be performed within 4 h with little preparation as lyophilized cells are always at the ready, and a multitude of samples can be assayed easily in the 96-well plate assay format. Being at least as sensitive, but faster and better suited for high-throughput analysis, whole-cell biosensors have the potential to displace growth inhibition assays as the favored method for TC residue screening.

ABBREVIATIONS USED

CTC, chlortetracycline; DC, doxycycline; EDTA, ethylenediaminetetraacetic acid; LB, Luria−Bertani broth; LBAmp, LB supplemented with 100 μg mL^{-1} ampicillin; LBPB, LB phosphate-buffered (100 mM) to pH 6; MRL, maximum residue limit; OTC, oxytetracycline; PMB, polymyxin B; TC, tetracycline.

ACKNOWLEDGMENT

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