ChemComm

COMMUNICATION

Cite this: *Chem. Commun.*, 2015, **51**, 5917

Received 2nd February 2015,
Accepted 23rd February 2015

DOI: 10.1039/c5cc00981b

www.rsc.org/chemcomm

A sensitive whole-cell biosensor for the simultaneous detection of a broad-spectrum of toxic heavy metal ions†

S. Cerminati, F. C. Soncini and S. K. Checa*

Bacterial biosensors are simple, cost-effective and efficient analytical tools for detecting bioavailable heavy metals in the environment. This work presents the design, construction and calibration of a novel whole-cell fluorescent biosensory device that, simultaneously and with high sensitivity, reports the presence of toxic mercury, lead, cadmium and/or gold ions in aqueous samples. This bio-reporter can be easily applied as an immediate alerting tool for detecting the presence of harmful pollutants in drinking water.

Mobilization and dissemination of toxic heavy metals into the environment has increased in the last few centuries mainly as a consequence of industrialization and improper waste disposal, particularly in regions where these anthropogenic activities are poorly controlled. Their harmfulness derives from their high reactivity damaging cellular components.[1] Besides acute intoxication, long-term exposure causes different and varying degrees of illness in humans and affects biodiversity,[2–4] being a matter of world-wide concern. In particular, the World Health Organization (WHO) has positioned lead (Pb), mercury (Hg) and cadmium (Cd) among the top ten more dangerous chemicals to human health when present in drinking water.[6] Thus, there is a need for tools that allow a rapid and economical screening of these intoxicants in the environment. In this sense, biosensors emerge as appealing low-cost alternatives to spectroscopic methods such as ICP and AAS, the analytical techniques routinely used worldwide to identify and quantify these pollutants.[7–9] Whole-cell metal biosensors are microorganisms, usually bacteria, genetically engineered to couple metal detection by a sensor protein with an output that can be easily recorded. Most designed metal-responsive biosensors are based on bacterial metal stress signal/transduction pathways.[7,10] Their main advantage is their ability to report only the fraction of the metal that is readily available to interact with the biota (bioavailable), providing direct estimation of toxicity, in contrast to other analytical techniques that quantify the total amount of metal present in the sample.[7,8] In addition, bacterial biosensors can be easily applied to the construction of portable detection instruments to perform *on site* monitoring.[11–14]

We have previously employed the regulatory circuit controlling gold resistance in *Salmonella enterica*[15] to generate the first whole-cell biosensor to selectively detect bioavailable gold (Au) with high sensitivity.[5] In this device, GolS, a sensor/regulatory protein of the MerR family, detects soluble, cytoplasmic Au(I)‡ activating its own expression as well as the expression of the reporter protein, *Aequorea victoria*'s green fluorescent protein (GFP), whose gene was in a plasmid, encoded under the control of the GolS-dependent *golB* promoter. The optimized performance and the modular design of the Au biosensor platform[5,10] allow the modification of its modules to generate new analytical tools for risk assessment. In this sense, we modified the metal binding site of the GolS sensor to expand the spectrum of uncomplexed metal ions detected.[16,17] Surprisingly, with single amino acid substitution at position 77 ($GolS_{S77C}$, see the inset in Fig. 1A) we were able to generate a variant that also responds to Hg(II) without decreasing its ability to detect Au ions.[18] Furthermore, in a *Salmonella* strain lacking the main Zn(II) exporter ZntA, this variant also activates the expression of its target genes in the presence of other divalent metal ions such as Zn(II), Pb(II) and Cd(II). The ability of $GolS_{S77C}$ to respond to such a variety of metal ions is rather an exception, as all characterized sensor proteins detect only one or just a group of metal ions with the same charge and/or similar coordination chemistry, and no one is able to respond simultaneously to the harmful Hg, Pb and Cd ions.[19]

We used the $GolS_{S77C}$ variant to obtain an efficient whole-cell biosensor for the detection of a broad-spectrum of toxic heavy metals. First, we validated the fluorescent biosensor platform for reporting the presence of different metal ions in the culture medium in the original *gol*-resident *S. enterica* sv. Typhimurium 14028 strain. A 14028s derivative carrying the $golS_{S77C}$ gene replacing the wild-type *golS* copy in the chromosome and deleted in the *zntA* gene was transformed with the GolS-dependent pPB-GFP reporter plasmid[5] (Fig. 1A). Then, the

Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR), Ocampo y Esmeralda, 2000-Rosario, Argentina. E-mail: checa@ibr-conicet.gov.ar

† Electronic supplementary information (ESI) available: Supporting Fig. S1–S5, Tables S1–S4 and Experimental procedures. See DOI: 10.1039/c5cc00981b

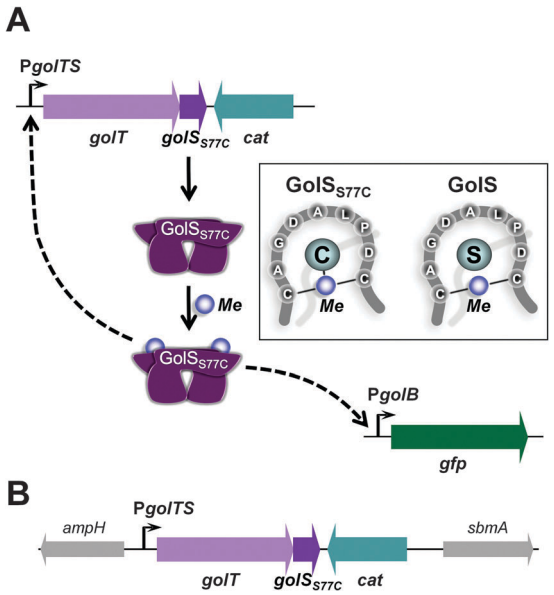

Fig. 1 The GolS$_{S77C}$-based biosensor platform. (A) The sensor protein GolS$_{S77C}$ is expressed from its chromosomally encoded gene in an operon with *golT* encoding the P_{1B}-type Au(I) transporter (STM14_0413-STM14_0412). Upon detecting the inducer metal ion (the input signal) at the bacterial cytoplasm, the sensor GolS$_{S77C}$ activates its own expression as well as the expression of GFP encoded in the reporter pPB-GFP plasmid providing the fluorescence output. The inset schematizes the metal binding domain in the native GolS and in the GolS$_{S77C}$ variant. The S–C replacement is highlighted. (B) Genetic organization of the site chosen for the insertion of the *golTS$_{S77C}$-cat* locus in the *E. coli* chromosome. Co-expression of GolT improves biosensor performance for Au detection in the *E. coli* chassis.[5]

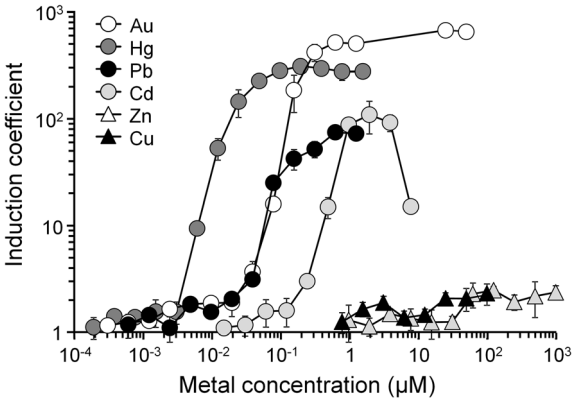

Fig. 2 Dose–response curves of the *E. coli* GolS$_{S77C}$-based biosensor to different metal ions. The response to $KAu(CN)_2$ (Au), $HgCl_2$ (Hg), $Pb(NO_3)_2$ (Pb), and $CdCl_2$ (Cd); $ZnCl_2$ (Zn) or $CuSO_4$ (Cu) was plotted against the final concentration of metal in the SM9 culture medium. Induction coefficient, IC, is the ratio between the normalized fluorescence measured in the presence *versus* the absence of a metal. The data represent the mean ± SD of at least five independent measurements done in triplicate.

cells were grown in SM9 minimal medium until the mid-exponential phase followed by a 3 h incubation with increasing concentrations of different metal ions to evaluate the sensor's response (see ESI† for details). Emitted fluorescence increased more than 170-fold in the presence of Au, Hg, Pb or Cd salts. Cu or Zn rendered less than 3- or 7-fold increase in fluorescence (Fig. S1A, ESI†), while no statistically significant induction was observed in the presence of Ni ions, which was expected not to be detected by the sensor protein. Similar results were obtained in rich culture media (data not shown). The response of the *Salmonella* biosensor to the toxic metals could also be evidenced by diffusion assays in agar plates or by fluorescence microscopy even when these metal ions are present in mixtures (Fig. S1B, ESI† and data not shown).

The biosensor platform was transferred to a laboratory *Escherichia coli* strain, a bacterium widely used as a microbial chassis in whole-cell biosensor designing.[20] To optimize metal determinations, the sensor protein has to be expressed from a single chromosomal copy and under the control of its own promoter. This guarantees negligible GolS$_{S77C}$ levels under non-inducing conditions, essential to attain a maximal metal discrimination index.[5] Because *E. coli* lacks the *gol* locus,[15] a single copy of the engineered *golT-golS$_{S77C}$ Salmonella* operon, including the native GolS-dependent *golTS* promoter, was introduced into the chromosome of the deleted *E. coli* MC1061 strain of the *zntA* gene (Fig. 1B). Details of the constructions of the strain are provided as ESI.† The transgenic *E. coli* cells were transformed with the pPB-GFP reporter plasmid (Fig. 1A) to evaluate the response to different metal salts. As shown in Fig. 2, fluorescence greatly increased in the presence of Au, Hg, Pb or Cd salts added to the culture medium, but not in the presence of Cu or Zn ions. When the dose–response curves obtained for the different metals in the *E. coli* and the *Salmonella* chassis are compared, we observed a similar pattern of induction, although the levels of maximal activation achieved were significantly lower in *E. coli* than in the original host, particularly for Pb(II), Cd(II) or Zn(II) ions (Fig. 2 and Fig. S1, ESI†), probably reflecting differences in permeability or handling of these metals between the tested bacterial species.

The dose–response curve for each of the inducer metal (Au, Hg, Pb and Cd) was fitted to a derived transfer function (see ESI† for details). The curves and the calculated parameters are shown in Fig. S2 and Table S1 (ESI†), respectively. We use these data and the equations detailed in the ESI† to estimate the limit of determination (LOD) and the concentration at which maximal induction is reached (CMI) for each metal. The *E. coli* GolS$_{S77C}$-based biosensor detected Au(I) with high sensitivity (LOD = 42.7 nM or 8.4 μg L^{-1}), similar to the previously reported values for the GolS-based Au biosensor.[5] Fluorescence increased up to 1.5 μM $KAu(CN)_2$, concentration at which the CMI is reached (Fig. S2 and Table S1, ESI†). The designed biosensor platform also detected Hg or Pb ions with calculated LOD values of 4.4 nM (0.9 μg L^{-1}) or 39.6 nM (8.2 μg L^{-1}), respectively, values that are near or below the safety levels in drinking water (1–6 μg L^{-1} for Hg and 10–15 μg L^{-1} for Pb) recommended by WHO[6] and other governmental protection agencies worldwide. CMI was obtained at 0.15 μM Hg(II) or 0.29 μM Pb(II). Cd(II) was detected with a sensitivity estimated in 283.9 nM (31.9 μg L^{-1}), reaching the CMI at 2.5 μM $CdCl_2$. Higher concentrations induced a sharp decrease in fluorescence, probably because of metal toxicity (Fig. S2, ESI†). Although the toxic effects of Cd and Pb were apparently less severe in *Salmonella*, no substantial differences were observed in sensitivity and the global performance of the designed biosensor platform for detecting Au, Hg, Pb or Cd ions between the two microbial chassis tested (Fig. S2 and S3, Tables S1 and S2, ESI†).

The above results highlight the high sensitivity of the *E. coli* $GolS_{S77C}$-based fluorescent biosensor for detecting bioavailable Hg, Pb or Cd ions in liquid samples under laboratory conditions. Thus, we propose that this non-selective biosensor can be applied as a primary warning tool for reporting the presence of these pollutants in water samples. In order to test this, a contaminated aqueous environment was mimicked by adding known concentrations of different metal ions to drinking water samples (taken directly from the tap) using two different protocols. In the first, we supplemented tap water with different mixtures of inducer (Au, Hg, Pb, Cd) or non-inducer (Zn, Cu) metal salts (Fig. 3). In the second, we supplement tap water with a fixed concentration of Au, Hg, Pb or Cd and with varying amounts of one or the other inducer metals (Fig. S4, ESI†). The artificially contaminated water samples were mixed in a 1 : 1 ratio with a mid-log phase culture of the biosensor bacteria, and incubated for 3 more hours under the same conditions (see ESI† for details). The increase in fluorescence observed for the samples supplemented with only one metal was almost identical to the response observed when the same amount of metal in a small volume (1 : 100 ratio) was added to the culture medium (Fig. 2, 3 and Fig. S4, ESI†), validating the testing protocols employed. In samples supplemented with more than one metal, the response was clearly additive and correlated well with the total amount of inducer metals present in the sample (Fig. 3 and Fig. S4, ESI†). In fact, no interference between these metals was observed at least when they are present at sub-saturating concentrations in the mixtures. Besides, the levels of emitted fluorescence were poorly affected by the presence of metals that do not act as inducers (Cu and/or Zn), even when these pollutants were added in excess (Fig. 3). The ability of the biosensors to report metal pollution in water was also validated by qualitative assays such as those shown in Fig. S1, ESI† (data not shown).

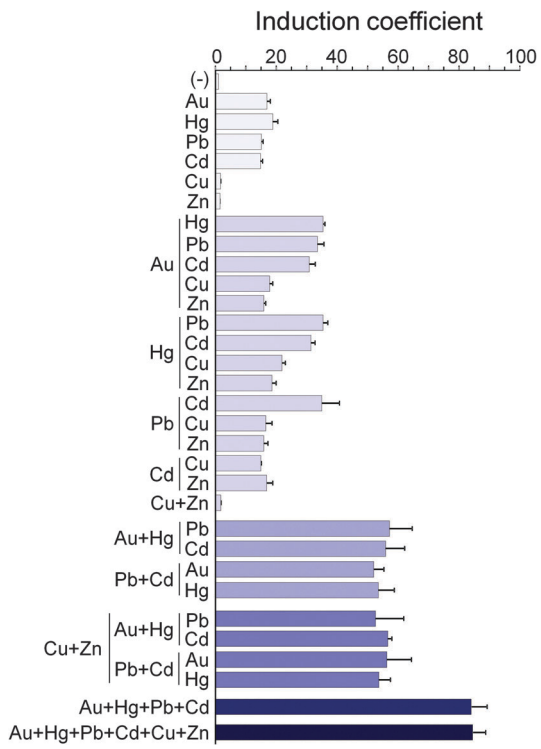

Fig. 3 Analysis of artificially contaminated tap water samples using the *E. coli* $GolS_{S77C}$-based biosensor. Tap water was supplemented with the indicated metal salts to reach a concentration in the final mixture of 80 nM $KAu(CN)_2$ (Au), 8 nM $HgCl_2$ (Hg), 80 nM $Pb(NO_3)_2$ (Pb), 500 nM $CdCl_2$ (Cd); 20 μM $CuSO_4$ (Cu) or 80 μM $ZnCl_2$ (Zn). The concentration of inducer metals to be tested was selected from the dose–response curves of Fig. 2 in order to produce less than 20% change in fluorescence, so they do not saturate the detection system when the four inducer metals are combined in a sample. The data represent the mean ± SD of IC values from at least four independent measurements done in triplicate.

We have previously shown that the low capability of GolS to respond to Cu or Ag ions resides in its metal binding loop and that an engineered GolS mutant with the loop of the Cu/Ag/Au sensor CueR ($GolS_L$) induces its reporter genes also in the presence of these metal ions.[16,17] To expand the spectrum of toxic metals detected, we further engineered $GolS_{S77C}$, replacing its metal binding loop (between C112 and C120) by the loop of CueR (Fig. S5A, ESI†). The reporter gene in the strain with this new GolS variant, $GolS_{S77C+L}$, was induced more than 70- or 45-fold in the presence of either 100 μM $CuSO_4$ or 10 μM $AgNO_3$, respectively (Fig. S5B, ESI†), while conserving the original $GolS_{S77C}$-based biosensor response to Au or Hg ions. This highlights the versatility of the GolS-based biosensor platform in the development of new and efficient metal biodetection tools.

The above results demonstrate the capability of the $GolS_{S77C}$-based platform to report simultaneously and with high sensitivity the presence of bioavailable Hg, Pb, Cd and/or Au in aqueous samples, integrating signal detection into a single and easily quantifiable output, and serve as a proof of concept for its practical application in monitoring heavy metal pollution. Without the identification of the pollutant, this device can be employed as an immediate screening tool to alert about the presence of harmful heavy metals in a sample, bypassing the need for an array of different biosensors.[21–23] Besides, this broad-spectrum metal biosensor can be applied alone or in combination with biosensors designed to detect other pollutants in the construction of portable analytical tools or instruments to perform on-site determinations.[11–13]

This work was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica and from the National Scientific and Technical Research Council (CONICET) to S.K.C., and F.C.S. S.C. is a fellow of the CONICET. S.K.C. and F.C.S. are career investigators of CONICET. F.C.S. is also a career investigator of the Rosario National University Research Council (CIUNR).

Notes and references

‡ Extracellular Au(III) is immediately reduced to Au(I) once inside the bacterial cytoplasm where it is detected by the GolS sensor.[14]

1 J. A. Lemire, J. J. Harrison and R. J. Turner, *Nat. Rev. Microbiol.*, 2013, **11**, 371–384.
2 T. W. Clarkson, L. Magos and G. J. Myers, *N. Engl. J. Med.*, 2003, **349**, 1731–1737.
3 K. Peters, M. Bundschuh and R. B. Schäfer, *Environ. Pollut.*, 2013, **180**, 324–329.
4 A. Neal and T. Guilarte, *Mol. Neurobiol.*, 2010, **42**, 151–160.
5 S. Cerminati, F. C. Soncini and S. K. Checa, *Biotechnol. Bioeng.*, 2011, **108**, 2553–2560.

6 World Health Organization, Guidelines for drinking-water quality, World Health Organization, Geneva, 4th edn, 2011.
7 A. Hynninen and M. Virta, *Advances in Biochemical Engineering/Biotechnology*, 2010, **118**, 31–63.
8 L. T. Bereza-Malcolm, G. Mann and A. E. Franks, *ACS Synth. Biol.*, 2014.
9 H. N. Kim, W. X. Ren, J. S. Kim and J. Yoon, *Chem. Soc. Rev.*, 2012, **41**, 3210–3244.
10 S. K. Checa, M. D. Zurbriggen and F. C. Soncini, *Curr. Opin. Biotechnol.*, 2012, **23**, 766–772.
11 J. R. van der Meer and S. Belkin, *Nat. Rev. Microbiol.*, 2010, **8**, 511–522.
12 A. de las Heras and V. de Lorenzo, *Anal. Bioanal. Chem.*, 2011, **400**, 1093–1104.
13 A. Roda, L. Cevenini, E. Michelini and B. R. Branchini, *Biosens. Bioelectron.*, 2011, **26**, 3647–3653.
14 K. Siegfried, C. Endes, A. F. M. K. Bhuiyan, A. Kuppardt, J. Mattusch, J. R. van der Meer, A. Chatzinotas and H. Harms, *Environ. Sci. Technol.*, 2012, **46**, 3281–3287.
15 S. K. Checa and F. C. Soncini, *Biometals*, 2011, **24**, 419–427.
16 S. K. Checa, M. Espariz, M. E. Pérez Audero, P. E. Botta, S. V. Spinelli and F. C. Soncini, *Mol. Microbiol.*, 2007, **63**, 1307–1318.
17 M. M. Ibánez, S. Cerminati, S. K. Checa and F. C. Soncini, *J. Bacteriol.*, 2013, **195**, 3084–3092.
18 M. M. Ibáñez, S. K. Checa and F. C. Soncini, *J. Bacteriol.*, 2015, DOI: 10.1128/JB.02565-14.
19 A. J. Guerra and D. P. Giedroc, *Arch. Biochem. Biophys.*, 2012, **519**, 210–222.
20 J. Robbens, F. Dardenne, L. Devriese, W. De Coen and R. Blust, *Appl. Microbiol. Biotechnol.*, 2010, **88**, 1007–1025.
21 S. Melamed, T. Elad and S. Belkin, *Curr. Opin. Biotechnol.*, 2012, **23**, 2–8.
22 S. Jouanneau, M.-J. Durand, P. Courcoux, T. Blusseau and G. Thouand, *Environ. Sci. Technol.*, 2011, **45**, 2925–2931.
23 B. Wang, M. Barahona and M. Buck, *Biosens. Bioelectron.*, 2013, **40**, 368–376.

