Danio rerio ABC transporter genes abcb3 and abcb7 play a protecting role against metal contamination.

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Short title: Zebrfish ABCB transporters protect against metal contamination.

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Abstract

ATP-binding cassette (ABC) proteins are efflux transporters and some of them are involved in xenobiotic detoxification. The involvement of four zebrafish ABC transporters in cadmium, zinc and mercury detoxification was characterised in a metal hypersensitive mutant of Escherichia coli. The E. coli tolC mutant expressing ABCB3 or ABCB7 transporters exhibited higher survival ratios and lower metal accumulation under metal exposure condition than controls. For instance, in presence of 8 and 10 µM of HgCl₂, the survival ratios of bacteria expressing ABCB3 were 4 and 6-times higher than control while mercury concentrations were 2.5 and 2-times lower than in control. This work provides new data on the function of zebrafish ABCB3 and ABCB7 transporters, and highlights their significance in metal detoxification.
1. Introduction

ATP-binding cassette (ABC) transporters are membrane proteins distributed among eight subfamilies A to G (Dean et al., 2001) and H (Popovic et al., 2010) based on structural arrangements and phylogenetic analyses. These efflux pumps carry various compounds across biological membrane including phospholipids, ions, peptides, steroids, polysaccharides amino acids, organic anions, bile acids, drugs and other xenobiotics. Mitochondria comprise up to four ABC systems, ABCB6, ABCB7/ATM1, ABCB10/MDL1, and ABCB8. These half-transporters, which assemble into homodimeric complexes, are involved in biogenesis of cytosolic iron–sulfur clusters, heme biosynthesis, iron homeostasis (Zutz et al., 2009), protection against oxidative stress (Liesa et al., 2012) and multidrug resistance (Elliott and Al-Hajj, 2009). Multidrug resistance is associated to overexpression of ABC transporters belonging to B (MDR1), C (MRP1 and MRP2) and G2 (BCRP) subfamilies (Leslie et al., 2005). Some ABC transporters carry toxic compounds and their metabolites out of the cell through binding and hydrolyzing ATP and play an important role in detoxification.

ABC transporter genes have been identified in fish species (Liu et al., 2013; Lončar et al., 2010; Tutundjian et al., 2002; Zucchi et al., 2010). Transcriptional responses of ABC transporter genes belonging to B and/or C sub-families have been studied in zebrafish (Danio rerio) (Bresolin et al., 2005), turbot (Scophthalmus maximus) (Tutundjian et al., 2002), rainbow trout (Oncorhynchus mykiss) (Zaja et al., 2008; Lončar et al., 2010), red mullet (Mullus barbatus) (Sauerborn et al., 2004), Nile tilapia (Oreochromis niloticus) (Costa et al., 2012) and rock cod (Trematomus bernacchii) (Zucchi et al., 2010). The identification of 50 ABC transporter genes was recently achieved by phylogenetic analyses in catfish (Liu et al., 2013). Proteins of the B subfamily have been identified in rainbow trout (Sturm et al., 2001), killifish (Fundulus heteroclitus) (Cooper et al., 1999), turbot (Tutundjian et al., 2002) and the rock cod (Zucchi et al., 2010). Finally, mRNA and proteins of ABC transporter have also been identified and
characterised in fish cells (Zaja et al., 2007, 2008, 2011). Transcriptional changes of ABCB1 and ABCC2 transporter genes have been detected in liver of rock cod after intraperitoneal injection of Cd, stimulating an acute exposure event (Zucchi et al., 2010) and in PLHC-1 cells exposed to Hg, Cd, As and Cr (Della Torre et al., 2012). The transcriptional response of abcb1, abcc2, and abcg2 genes was modified in liver of killifish exposed to a marine site polluted by polycyclic aromatic hydrocarbons, as compared to fish from a clean environment (Paetzold et al., 2009). The abcb1 gene is absent in the zebrafish genome. Zebrafish ABCB4 and ABCB5 are structurally similar to mammalian ABCB1, and it has been shown that ABCB4 transporter, but not ABCB5, conferred resistance of embryos to ABCB1 substrates (Fischer et al., 2013).

Four zebrafish ABC genes responding to metal exposure in adult zebrafish have been selected, and their cDNAs have been heterologously expressed in a model cell reactor, the bacterium Escherichia coli, in order to assess the possible resistance conferred by those zebrafish ABC transporters against cadmium, zinc and mercury contaminations. E. coli does not possess MDR, MRP or BCRP-like ABC transporter genes in its genome, so that any beneficial action against metal of zebrafish cDNAs expressed in that bacterium would indicate their potential protecting role in fish.

2. Material and methods

2.1 Selection of zebrafish genes encoding ABC transporters for heterologous expression in tolC mutant

We selected them without any preconceived idea about their function, because 1/ the function of many ABC genes is only inferred from sequence alignment and not from thorough biochemical studies, and 2/ some ABC transporters were found to display various functions besides their historically discovered one. Indeed, 1/ the human cystic fibrosis transmembrane conductance regulator, CFTR, historically known to pump out chloride anions has recently been
shown to pump out glutathione (Gould et al., 2012); 2/ CFTR has also been demonstrated to conduct ATP movements (Reisin et al., 1994); 3/ Murine and drosophila P-gp homologues have been shown to serve as ATP-conducting channels in the plasma membrane (Abraham et al., 1993; Bosch et al., 1996); 4/ The bacterial P-gp homologue LmrA (van Veen et al., 1998) protected bacteria not only against sodium laurate toxicity, ethanol, and wine shock, but also against sodium chloride shock (Bourdineaud et al., 2004). It was further confirmed that indeed LmrA can pump out salt (Velamakanni et al., 2009); 5/ MDR1 protein also displays a chloride-channel activity and regulates the cell volume (Valverde et al., 1992; Valverde et al., 1996); 6/ Overexpression of CFTR leads to a multidrug resistance phenotype (Wei et al., 1995) and induction by antitumoral drugs of hMDR1 and hMRP1 complements CFTR function (Lallemand et al., 1997). These results raise the possibility that ABC transporters may be interchanged, as outlined by the complementary patterns of expression of the CFTR and hMDR1 genes observed in vivo (Breuer et al., 1993). The common functional features in ABC transporters are likely to be explained by an early evolutionary event. ABC genes may have evolved separately and acquired a more specialized activity, while maintaining their general function with low efficiency.

Therefore our selection was not guided by the supposed function of ABC transporters but rather by their pattern of expression in response to metals in zebrafish tissues (Bourdineaud et al., 2015). For instances abcb4, abcb11b and abcc7 members were excluded because their level of expression was found too weak in gills (despite the fact abcb4 is expressed in zebrafish embryo; Fischer et al., 2013). The expression of abcb4, abcb10, abcb11b, abcc6a, abcc7 and abcc12 members were also found too weak in brain and liver. The following genes were selected for heterologous expression in E. coli: abcb3 because of its induction by cadmium exposure in liver; abcb3l1 because of its induction by cadmium exposure in brain; abcb7 because of its induction by zinc exposure in muscles; and abcb8 because of its induction by cadmium exposure in gills and zinc exposure in muscles and gills (Bourdineaud et al., 2015). In addition, the abcb3l1 gene
had been found up regulated in gills, muscles, digestive tract and brain of zebrafish after 3 days of exposure to the contaminated water of the Riou-Mort River (in the south west of France, the Lot River and one of its tributaries, the Riou-Mort, are polluted by Cd and Zn released by an old industrial Zn factory) (Orieux et al., 2011). Besides, we found it relevant to include \textit{abcb3} and \textit{abcb3ll} genes because they actively transport peptides from the cytosol to the ER lumen and display structural similarities with other transporters. Therefore we thought that they might be involved in the transport of peptide-metal complexes. Another criterion of selection was the length of the expressed transporter: we limited its size to 715-744 amino-acids to avoid peptide folding problems frequently encountered in \textit{E. coli} with big proteins. Indeed, only half-transporters are encountered in bacteria.

\textit{2.2 Heterologous expression of ABC transporters}

Specific primer pairs were designed (Table 1). The Shine-Dalgarno sequence and a stop codon were added in the 5’ and 3’ moiety of forward and reverse primers, respectively. All transcripts were successfully amplified using the Expand enzyme “Expand Long Template PCR System” (Roche). PCR products were visualized on 1% agarose gel containing crystal violet in order to prevent DNA from UV exposure required when using ethidium bromide. DNA bands were extracted and purified using the “TOPO XL PCR Cloning” kit (Invitrogen). Their integrity was checked on 1% agarose gel. One µL vector was mixed with 4 µL of PCR products and incubated at room temperature for 5 min and transformed in TOP 10 \textit{E. coli}. Bacteria were then spread on agarose plates containing kanamycine (50 µg/mL). PCRs were performed to check the size and the orientation of the insert using internal primers as well as plasmid primers (M13 and T7). Orientation of the inserts was opposed to \textit{lacZ} promoter in plasmids containing ADNc of \textit{abcb3}, \textit{abcb8} and \textit{abcb3ll}. This suggests that the overexpression of these transporter genes are toxic in \textit{E. coli} and that only a moderate expression is compatible with bacterial life. Only \textit{abcb7}
displayed both orientations. Moreover, tolC mutant containing abcb7 cDNA under lacZ control exhibited a slower growth than that transformed with the abcb7 cDNA in the reverse orientation. Thus, the latter construction was used to perform experiments. Therefore, the system of expression used in this study is deliberately devoid of promoter in order to reach a low abundance of transporter molecules within cells, in order to get rid off possible disturbance, toxicity and inclusion bodies formation. Plasmids were extracted using a lysis protocol (Berghammer and Auer, 1993), and transferred in mutant bacteria E. coli tolC CS1562 (F-λ- tolC6::Tn10 his leu proA argT his thi galK lacY trpE non mtl xyl ara rpsL sup+) (Austin et al., 1990) in order to study the potential resistance to metal exposure conferred by ABC transporters.

2.3 Inhibitory concentrations

Concentrations inhibiting growth at 50% (IC₅₀) were determined for zinc, mercury and cadmium in order to define the exposure concentrations to use regarding a potential influence of ABC transporters in metal detoxification. Overnight tolC bacterial cultures hosting control or cDNA-containing plasmid were diluted to an OD₆₀₀ of 0.01 into LB medium containing kanamycin (50 µg/mL) and different concentrations of mercury (from 0 to 20 µM of HgCl₂), zinc (from 0 to 2 mM of ZnCl₂) and cadmium (from 0 to 2 mM of CdCl₂). After 16 h of incubation at 28°C cell growth was monitored by spectrometry (absorbance at 600 nm) and IC₅₀ were calculated by plotting the observed optical density versus the concentrations of metals in the medium. At 50 % of growth inhibition, the corresponding concentration was chosen to give the IC₅₀. The mean IC₅₀ was obtained from 3 independent plots. Selected from these IC₅₀ values, working exposure concentrations were 0.55 and 0.8 mM for ZnCl₂, 8 and 10 µM for HgCl₂ and 0.4 and 0.6 mM for CdCl₂.

2.4 Bacterial survival quantification
Overnight cultures were diluted into fresh LB medium (1% v/v) containing kanamycin and grown up to an OD₆₀₀ of 0.1. Each culture was divided into seven: one control and six exposed to the targeted metal concentrations (ZnCl₂: 0.55 and 0.8 mM, HgCl₂: 8 and 10 µM and CdCl₂: 0.4 and 0.6 mM). Cultures were exposed during 3h at 28°C and 100 µL of each culture were used to perform serial 10-fold dilutions and each was spread on LB agar plates. Colonies were counted after overnight incubation. Survival rates are the ratio between the number of colonies after exposure to metals on those counted without exposure to metals.

2.5 Quantification of metal concentrations in bacteria

After 3 h of incubation as described previously, cells from 3 mL cultures were collected by centrifugation (5 min, 6000 g, 4 °C) and washed with a buffer containing 10 mM Tris-Cl pH 7.4 and 0.15 M NaCl. Pellets were mineralized in 3 mL of concentrated nitric acid (pure HNO₃ 60%, v/v) at 100 °C for 3 h in a pressurized medium (borosilicate glass tube). Samples were diluted in 18 mL of ultrapure water (MilliQ plus) and analyzed by atomic absorption spectrophotometry. The Cd determinations were performed with an atomic absorption spectrophotometer (M6 Solaar AA, Thermo Elemental) equipped with a graphite tube atomizer (GF95 Graphite Furnace). The detection and quantification limits were 0.44 nmol Cd/L and 2.6 pmol Cd/10⁹ bacterial cells. Zinc concentrations for water and digested tissue samples were determined by flame atomic absorption spectrophotometry (AAS) (Varian AA 220 FS). The detection and quantification limits were 0.15 µmol Zn/L and 0.9 nmol Zn/10⁹ bacterial cells. The analytical methods were simultaneously validated for each sample series by the analysis of standard biological reference materials (TORT-2, lobster hepatopancreas; DOLT-2, dogfish liver; National Research Council of Canada, Ottawa). Total Hg concentration was determined by flameless AAS directly on bacterial samples (Leco Ama 254). The detection limit was 0.01 ng of Hg.
2.6 Statistical analyses

As the normality of the residuals wasn’t validated, non-parametric tests were selected. After a significant Kruskall-Wallis test, the Mann-Whitney U test was used to evaluate significant difference between results from an exposed condition versus the corresponding control one for survival ratios, metal quantification and IC50 analyses (*: \( p < 0.05 \)). Both tests were performed using R (version 3.0.2) coin package.

3. Results

3.1 ABC transporter proteins confer metal resistance to E. coli

The \( tolC \) mutant, which is hypersensitive to metals (Achard-Joris et al., 2005), was transformed with a control plasmid or plasmids containing cDNA of ABC transporters \( abcb3, \ abcb7, \ abcb3l1 \) and \( abcb8 \), and then tested for its resistance to metal exposure (\( \text{CdCl}_2, \text{ZnCl}_2 \) and \( \text{HgCl}_2 \)). When transformed with plasmids containing \( abcb3 \) and \( abcb7 \) bacteria displayed a significant increase of 50% of growth inhibitory concentration (IC\(_{50}\)) values as compared to control bacteria for every metal (Table 2). In contrast, there were no differences in cell growth for bacteria containing \( abcb3l1 \) or \( abcb8 \) cDNAs as compared to control.

3.2 Expression of \( abcb3 \) and \( abcb7 \) cDNAs result in a lower metal accumulation in bacteria

\( E. \ coli \ tolC \) mutant expressing ABCB3 or ABCB7 transporters exhibited higher survival ratios and lower metal accumulations under metal exposure condition than controls (Table 2 and Figure 1). For instance, in presence of 8 and 10 \( \mu \text{M} \) of \( \text{HgCl}_2 \), the survival ratios of bacteria expressing ABCB3 were 4- and 6-times higher than control while mercury concentrations were 2.5- and 2-times lower than in control. The same pattern of resistance associated with a decreased accumulation of metal was observed for cadmium and zinc exposures as well as for bacteria
expressing ABCB7 (Table 2 and Figure 1). When E. coli tolC mutant expressed ABCB311 and ABCB8, survival ratios and metal accumulation levels were similar to control (Figure 1).

4. Discussion

cDNA of four transporter genes belonging to the sub-families ABCB and ABCC were expressed in tolC mutant, which is hypersensitive to metals (Achard-Joris et al., 2005; Achard-Joris and Bourdineaud, 2006). In the present study, we used this mutant as a simple reactor in which transporters were expressed in order to assess their ability to confer resistance to E. coli when exposed to cadmium, zinc and mercury. Previous studies evidenced that human MDR1 and two bacterial homologs, LmrA and OmrA, protected tolC mutant against Cd, Zn and Hg (Achard-Joris et al., 2005; Achard-Joris and Bourdineaud, 2006). ABCB3 and ABCB7 expression induced a survival increase varying between + 13 % and + 58 % and a decrease in metal concentration ranging between - 50 % to - 150 %. Numerous data in the literature, including those using a bacterial system like in the present work, are giving differences in the same range when dealing with the involvement of ATPase transporters in metal or xenobiotic detoxification. Indeed, 1/ The mortality of zebrafish embryos caused by 10 and 20 μM vincristine was increased from 6.5 to 26 % (+ 19.5 %) and from 29 to 39 % (+ 10 %) upon addition of 5 μM of the ABC transporters inhibitor cyclosporine A, respectively (Fischer et al., 2013); 2/ When using morpholino compounds to knock-down abcb4 gene, the mortality in presence of 2 μM vinblastine was increased from 51 % in controls up to 74 % in targeted embryos (+ 23 %) (Fischer et al., 2013); 3/ The addition of the MRP proteins inhibitor MK-571 to canine kidney cells (expressing MRP1 and MRP2) resulted in a decreased survival ratio from 71 down to 47 % (- 24 %) and from 54 down to 27 % (- 27 %), during exposure to 20 and 40 μM of HgCl2, respectively. The intracellular mercury simultaneously increased of from 1.26 up to 1.66 μg/mg protein (+ 32 %) when cells were exposed to 40 μM of HgCl2 (Aleo et al., 2005); 4/ The overexpression of abcc1
(mrp1) protected zebrafish embryos from acute toxicity of cadmium (100 μM) from 26 up to 41 % of survival (+ 15 %), mercury (3 μM) from 33 up to 52 % (+ 19 %), and arsenic (2 mM) from 45 up to 61 % (+ 16 %) (Long et al., 2011a); 5/ The overexpression of abcc2 (mrp2) decreased lead accumulation in zebrafish embryos exposed to 5 μM Pb from 145 to 87 nmol/g dry weight (- 40 %) (Long et al., 2011b); 6/ The overexpression of abcc5 (mrp5) protected zebrafish embryos from acute toxicity of cadmium (100 μM) from 58 up to 77 % of survival (+ 19 %) (Long et al., 2011c); 7/ The IC50 value for cadmium on wild-type zebrafish fibroblast cells was 71 ± 4 μM and 105 ± 7 μM for Cd-resistant cells overexpressing abcc2 and abcc4 genes (+ 49 %) (Long et al., 2011d); 8/ The expression of the PbtA transporter from Achromobacter xylosoxidans in E. coli decreased the accumulation of Cd, Pb and Zn (37, 13 and 20 %, respectively). The IC50 value for zinc increased from 78 to 134 μM (+ 72 %) upon expression of pbtA gene in E. coli (Hložková et al., 2013); 9/ The differences in IC50 values between a zntA mutant of Thermus thermophilus and a wild-type strain overexpressing ZntA were 33, 25 and 38 % after exposure to cadmium, copper and zinc (Schurig-Briccio and Gennis, 2012); 10/ The overexpression of the CzrB transporter from T. thermophilus in E. coli was associated with increased IC50 values from 1.6 up to 2.5 mM (+ 56 %) for zinc and 0.7 up to 0.9 mM (+ 28 %) for cadmium. The intracellular zinc concentration decreased from 75 down to 35 mg/g dry weight cells (- 53 %) after 1 h of incubation with 5 mM Zn (Spada et al., 2002); 11/ Arabidopsis thaliana plants expressing the E. coli ZntA transporter displayed resistance to 70 μM cadmium with increased fresh weight from 22 up to 38 mg/plant (+ 73 %), increased chlorophyll content from 16 to 21 mg/ml of plant extract (+ 31 %), and decreased cadmium concentration from 620 down to 355 nmol/mg dw (- 42 %) (Lee et al., 2003); 12/ The expression of the rice heavy-metal ATPase OsHMA9 in the E. coli copA mutant led to an increased IC50 value from 1.6 up to 3 mM CuSO4 (+ 87 %). Mutant plants knocked-out for oshma9 gene featured a greater sensitivity towards 300 μM copper with a plant height decreased from 11 down to 8 cm (- 27 %) and a fresh weight decreased from 110 down to
80 mg/plant (- 27 %) (Lee et al., 2007). To conclude, our bacterial tolC system is a relevant one to figure out the possible involvement of half ABC transporters in metal resistance.

The number of ABCB3 and ABCB7 molecules expressed per bacterial cell was estimated taking the zwf gene as a reference (encoding the glucose-6-phosphate dehydrogenase, G6PDH). The related specific primers have already been used and published (Kabir and Shimizu, 2003). Under non-exposed growth we found relative expressions of 0.29 ± 0.05 and 0.27 ± 0.04 for abcb3 and abcb7 cDNAs, respectively (n = 4, ± SD; a Roche LightCycler apparatus was used for qPCR analysis). The abundance of the zwf gene product, G6PDH, has been quantified to be 883 molecules per cell (Ishihama et al., 2008). If we accept the assumption according to which the rate of translation of the zwf RNA is equal to that of the abcb3 and abcb7 RNAs and the proteins half-lives similar, then the number of transporter molecules per cell is 256 ± 44 for ABCB3 and 238 ± 35 for ABCB7. We found herein that the expression of ABCB7 was responsible of the reduction of cadmium concentration from 41 down to 22 nmol/10^9 cells, making 114 x 10^{14} Cd ions pumped by 238 x 10^9 ABCB7 molecules for 3 hours yielding 4.4 ± 0.7 Cd ions/s/molecule. This is in accordance with ATP hydrolysis activity of metal ATPases such as E. coli ZntA (3.9 ATP/s/molecule; Sharma et al., 2000), T. thermophilus Zn^{2+}/Cd^{2+}-ATPase (2.4 ATP/s/molecule; Schurig-Briccio and Gennis, 2012) and even the human MDR1 (4.9 ATP/s/molecule; Loo et al., 2012). These results strongly suggest that ABCB3 and ABCB7 are involved in metal detoxification. Additionally, inductions of abcb3 gene in liver of fish exposed to cadmium and abcb7 gene in muscles of fish exposed to zinc corroborate their involvement in protection (Bourdineaud et al., 2015). The present study characterised for the first time the function of ABCB3 and ABCB7 transporters in metal detoxification. Indeed, these zebrafish proteins were classified among the ABCB subfamily on the basis of sequence alignment with counterparts from other species, but no data related to their genuine biochemical properties were known. Bacteria are lacking intracellular membranes whereas in eukaryotic cells, ABCB half transporters are
known to localise within such intracellular membranes questioning the role of those transporters in reducing cellular metal concentration. ABCB7 might well contribute to mitochondria detoxification by pumping divalent metals from the matrix out in the cytoplasm. Indeed, when cells are facing metal contamination, mitochondria are primary targets due to the developed transmembrane potential of around 120 mV. This creates a thermodynamic driving force attracting divalent metals within the negatively charged matrix so important that the theoretical ratio between divalent metal concentration within the matrix over the one in the cytoplasm at the Nernst equilibrium is equal to $e^k$ (with $k = 2F\Delta\Psi/RT$) giving a value of 8100. ABCB3 transporter equivalent to human transporters located within the endoplasmic reticulum and involved in peptide transport might as well be implicated in the transport of peptide-metal complexes, whenever a peptide containing a cysteinyI residue bound to a divalent metal ion is processed.

To summarise, the belonging of \textit{abcb3} an \textit{abcb7} genes to the ABC superfamily could only be deduced from sequence alignments, and our study introduces new information in the field of ABC transporters and sheds light on a new function of ABCB3 and ABCB7 zebrafish transporters, which was up to now ignored by protein data banks and literature.

\textbf{Acknowledgements}

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ABCB and ABCC transporter genes responding to cadmium and zinc contamination in zebrafish 


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Figure 1. Heterologously expressed ABCB3 and ABCB7 transporters protect *E. coli tolC* mutant against metals and are associated with a lower cell level of cadmium. *E. coli tolC* mutant was transformed with control plasmid or plasmids containing *abcb3*, *abcb3l1*, *abcb7*, and *abcb8* cDNAs. After 3 hours of incubation at 28°C with the indicated concentrations of HgCl$_2$ (A, D), CdCl$_2$ (B, E) and ZnCl$_2$ (C, F), the bacterial cultures were diluted and spread on agar plates in order to determine the survival ratio (A, B, C). The remaining cultures were used to quantify cell-associated metals (D, E, F).
<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Amplicon size</th>
<th>Primer sequences</th>
</tr>
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<tr>
<td>abcb3</td>
<td>ABCB3</td>
<td>2187 bp</td>
<td>^AACGGAGGTITAAAGATGATTCAGATCAGG&lt;sup&gt;a&lt;/sup&gt;&lt;br&gt;^CGTACCTAAGTCCTGTTGCG&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>abcb3l1</td>
<td>TAP2</td>
<td>2178 bp</td>
<td>^AGTTAGGAGCCGACCATGCGGAAGGTTTTG&lt;sup&gt;a&lt;/sup&gt;&lt;br&gt;^GGCCTACTGCGTTTTACGGTA&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>abcb7</td>
<td>ABCB7</td>
<td>2232 bp</td>
<td>^TCAACCAGGAGGTTCAGCATGCGCCCGCTCTT&lt;sup&gt;a&lt;/sup&gt;&lt;br&gt;^GGACTCAGCACGACAGCTTCC&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>ABCB8</td>
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<sup>a</sup>forward primer. <sup>b</sup>reverse primer.
Table 2. Median inhibiting concentrations (IC$_{50}$) of Zn, Hg and Cd in tolC mutant transformed with plasmids containing ABC transporter cDNA (mean ± SEM; n = 3, *: p < 0.05).

<table>
<thead>
<tr>
<th>ADNc</th>
<th>Protein</th>
<th>ZnCl$_2$ (mM)</th>
<th>HgCl$_2$ (µM)</th>
<th>CdCl$_2$ (mM)</th>
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<tr>
<td>control</td>
<td>none</td>
<td>0.63 ± 0.04</td>
<td>6.6 ± 0.4</td>
<td>0.59 ± 0.01</td>
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<tr>
<td>abcb3</td>
<td>ABCB3</td>
<td>* 0.96 ± 0.03</td>
<td>* 9.5 ± 0.3</td>
<td>* 0.82 ± 0.02</td>
</tr>
<tr>
<td>abcb3l1</td>
<td>TAP2</td>
<td>0.60 ± 0.03</td>
<td>7.1 ± 0.3</td>
<td>0.68 ± 0.04</td>
</tr>
<tr>
<td>abcb7</td>
<td>ABCB7</td>
<td>* 0.82 ± 0.06</td>
<td>* 9.7 ± 0.3</td>
<td>* 0.81 ± 0.02</td>
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<tr>
<td>abcb8</td>
<td>ABCB8</td>
<td>0.43 ± 0.03</td>
<td>6.0 ± 0.6</td>
<td>0.64 ± 0.04</td>
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</table>
Table 3. ABCB3 and ABCB7 transporters allow metal resistance in *tolC* mutant by decreasing the amount of bioaccumulated metals (mean ± SEM, *n* = 3, *: p < 0.05).

<table>
<thead>
<tr>
<th>Metal</th>
<th>Concentration</th>
<th>Metal accumulated (nmol/10⁹ bacteria)</th>
<th>Survival ratio (%)</th>
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<tr>
<td></td>
<td></td>
<td>ABCB3</td>
<td>ABCB7</td>
</tr>
<tr>
<td>HgCl₂</td>
<td>8 μM</td>
<td>*1.0 ± 0.1</td>
<td>*1.0 ± 0.1</td>
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<td></td>
<td>10 μM</td>
<td>*2.5 ± 0.1</td>
<td>*2.9 ± 0.3</td>
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<tr>
<td>CdCl₂</td>
<td>0.4 mM</td>
<td>9.1 ± 1.1</td>
<td>*6.6 ± 0.8</td>
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<tr>
<td></td>
<td>0.6 mM</td>
<td>*25 ± 2</td>
<td>*22 ± 2</td>
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<tr>
<td>ZnCl₂</td>
<td>0.55 mM</td>
<td>17 ± 4</td>
<td>16 ± 3</td>
</tr>
<tr>
<td></td>
<td>0.8 mM</td>
<td>*42 ± 7</td>
<td>*43 ± 2</td>
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