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ORIGINAL RESEARCH

# Early gene expression in *Pseudomonas fluorescens* exposed to a polymetallic solution

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**Abstract** The molecular response of *Pseudomonas fluorescens* cells exposed to a mixture of heavy metals remains largely unknown. Here, we studied the temporal changes in the early gene expression of *P. fluorescens* cells exposed to three doses of a polymetallic solution over two exposure times, through the application of a customized cDNA microarray. At the lowest metal dose (MD/4), we observed a repression of the Hsp70 chaperone system, MATE and MFS transporters, TonB membrane transporter and histidine kinases, together with an overexpression of metal transport (ChaC, CopC), chemotaxis and glutamine synthetase genes. At the intermediate metal dose (MD), several amino acid transporters, a response regulator (CheY), a TonB-dependent receptor and the *mutT* DNA repair gene were repressed; by contrast, an overexpression of genes associated with the antioxidative stress system and the transport of chelates and sulfur was observed. Finally, at the highest metal dose (4MD), a repression of genes encoding metal ion transporters, drug resistance and alginate biosynthesis was found, together with an overexpression of genes encoding antioxidative proteins, membrane transporters, ribosomal proteins, chaperones and proteases. It was concluded that *P. fluorescens* cells showed, over exposure time, a highly complex molecular response when exposed to a polymetallic solution, involving mechanisms related with chemotaxis, signal transmission, membrane transport, cellular redox state, and the regulation of transcription and ribosomal activity.

**Keywords** Exposure time · Metal toxicity · Toxicogenomics · Trace elements · Transcriptomics

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## Introduction

Heavy metal pollution is currently one of the most serious problems for the functionality of ecosystems. Heavy metals cannot be degraded and, therefore, accumulate in the environment; on the other hand, they present a high affinity for biomolecules with concomitant adverse effects for metal-exposed organisms. Nonetheless, along evolutionary history, bacteria have acquired tolerance to heavy metal exposure through a variety of strategies: regulation of metal uptake, active efflux, intracellular sequestration, etc. (Gadd 2010).

*Pseudomonas fluorescens* has been proposed as a model organism for the study of bacterial adaptation to harsh environmental conditions (Lemire et al. 2010).

Relevantly, many authors have found *P. fluorescens* as a perfect model species for the study of metal-induced bacterial responses (Sharma et al. 2006; Wasi et al. 2008, 2013; Sarma et al. 2010).

However, the mechanisms of metal toxicity and tolerance in bacteria are still not fully understood. Specifically, the molecular response of *P. fluorescens* cells exposed to a mixture of heavy metals remains largely unknown. Fortunately, recent advances in toxicogenomics and microarray technology are speeding up the discovery of gene regulation mechanisms in bacterial cells subjected to abiotic stresses. In particular, the development of microarray technology has facilitated the study of stress-induced metabolic responses in *Pseudomonas* (Reva et al. 2006; Teitzel et al. 2006; Thaden et al. 2010; Lee et al. 2014).

There are not many studies on the impact of mixtures of heavy metals on exposed organisms. Actually, most metal toxicology studies to date have dealt with the effects of one single heavy metal or, alternatively, a mixture of a few heavy metals. But polluted sites are frequently characterized by the simultaneous presence of many heavy metals, thus increasing the complexity of toxic responses in exposed organisms. Furthermore, the majority of studies have focused on "dose-effect" responses rather than on the effect of "exposure time". Accordingly, we published a study on the temporal changes in the early gene expression profiles of *Escherichia coli* cells subjected to three polymetallic treatments over different exposure times (Gómez-Sagasti et al. 2014). The aim of the current study was to complement such work by means of investigating the temporal changes in the early gene expression of another Gram-negative bacterium, i.e. *P. fluorescens*, exposed to three doses of a polymetallic solution over two exposure times (5 and 15 min), through the application of a customized complementary DNA (cDNA) microarray. To our knowledge, this is the first study on early transcriptional responses of *P. fluorescens* cells simultaneously exposed to a mixture of many heavy metals using microarray technology.

## Materials and methods

### *P. fluorescens* growth conditions

*P. fluorescens* ATCC 13525 strain, kindly provided by Dr. Iñigo Azúa (University of the Basque Country), was maintained in Luria broth (LB) liquid medium at 30 °C. All inoculations were made at 1 % volume from an overnight LB culture. Cycloheximide (100 mg $L^{-1}$) was added to the medium to prevent fungal growth.

### Effect of heavy metal dose on *P. fluorescens* growth

The effect of heavy metal dose on the growth of *P. fluorescens* cells subjected to the polymetallic treatments (see below) was quantified following Moore et al. (2005) as described in Gómez-Sagasti et al. (2014).

*P. fluorescens* cells were exposed to three doses of a polymetallic solution containing Ag(I), Pb(II), Cd(II), Cu(II), Ni(II) and Zn(II), as nitrate salts. Specifically, *P. fluorescens* cells were exposed to the following metal dose (MD): 10 μM Ag(I), 10 μM Pb(II), 10 μM Cd(II), 10 μM Cu(II), 500 μM Ni(II) and 300 μM Zn(II), following Moore et al. (2005) (although As was substituted by Pb since, in our region, Pb is a much more common soil pollutant than As). The effect of a fourfold higher (4MD) and a fourfold lower (MD/4) heavy metal dose on *P. fluorescens* cell growth was also quantified. Control cells were grown in the absence of heavy metals. Heavy metal-induced inhibition of cell growth (%) was determined according to Gómez-Sagasti et al. (2014).

### cDNA microarray studies

Under sterile conditions, *P. fluorescens* cells were grown at 30 °C in 250-mL Erlenmeyer flasks containing 45 mL of LB. Flasks were shaken at 180 rpm until mid-exponential phase ($OD_{595}$=0.45) when cell cultures (in duplicate) were supplemented with 5 mL of LB containing the required metal salt concentration, in order to obtain the metal doses described above (MD/4, MD, 4MD, control). For each treatment, a 10-mL sample was taken from the flasks after 5 and 15 min, respectively (i.e. two exposure times). Samples were stabilized by using RNAProtect Bacteria (Qiagen), and then, total RNA was isolated with the RNeasy Mini Kit (Qiagen), as described in Gómez-Sagasti et al. (2014). The quantity and quality of the extracted RNA was assessed using NanoDrop-1000 (NanoDrop Technologies) and Bioanalyzer 2100 (Agilent), respectively. RNA samples were stored at −80 °C until use.

The temporal changes in the early gene expression of *P. fluorescens* exposed to the polymetallic treatments were investigated through the application of our own

customized cDNA 8×15 K microarray (Design ID: 036764 Agilent, https://earray.chem.agilent.com/earray/). To this purpose, all known coding sequences from the transcriptome of *P. fluorescens* Pf0-1 found in the JCVI-CMR (Taxon ID 205922), DDBJ-GTPS (Pflu_PFO1: GIB00282CH01 CP000094) and JGI-IMG (Taxon ID 637000221) databases were used. For each target sequence, a probe was designed using Agilent's eArray. Probe sequences (60-mer) were selected according to eArray's Base Composition Methodology and synthesized on a microarray 8× 15 K platform using Sure-Print Technology (Agilent).

The impact of multiple-metal exposure on *P. fluorescens* gene expression was investigated by hybridization of fluorescently labelled (with Cy3) cDNA samples to our customized microarray. cDNA synthesis, labelling and hybridization were performed following Agilent's One-Color Microarray-Based Prokaryote Analysis—Fairplay III Microarray Labeling v. 1.3 protocol, as indicated in Gómez-Sagasti et al. (2014).

After hybridization, data were extracted using the Agilent Feature Extraction Software v. 10.7.3.1 (Agilent) following the GE1-107-Sep09 protocol, and then processed by GeneSpring GX 11.5.1 software (Agilent). Data were normalized using the quantile method (Bolstad et al. 2003) and centred by median. In order to simplify data handling, the average value of the signal intensity for each probe was transformed to $\log_2$. Using $\log_2$ signal values, the absolute fold change for each probe was calculated according to the following criteria (Leonhardt et al. 2004): if $\log_2$ signal value>0, fold change $= 2^{(\log_2 \text{ signal value})}$; if $\log_2$ signal value<0, fold change $= (-1) \times 2^{-(\log_2 \text{ signal value})}$. From here onwards, the term "gene" will be used to refer to "probes". Microarray data were deposited in the EMBL-EBI ArrayExpress, accession E-MTAB-3094.

## Statistical analysis of genes differentially expressed over exposure time

Within each heavy metal dose, statistically significant differences between exposure times (i.e. 5 vs. 15 min) were analysed using the Bayesian estimation of temporal regulation (BETR) ($p<0.01$) (Aryee et al. 2009), available in MultiExperiment Viewer (MeV) Open Source Software v. 4.7.1. Gene expression patterns were visualized by the *K*-means clustering method (Soukas et al. 2000) integrated in the MultiExperiment Viewer (MeV) Open Source Software v. 4.7.1. The measure of figure of merit (Yeung et al. 2001) was used to estimate an appropriate value for *K* (i.e. number of clusters). The clustering procedure was based on Pearson distance and used the average linkage method. Out of all the clusters, we selected only those clusters showing a progressive increase (overexpression trend, 5 min<15 min) or decrease (repression trend, 5 min>15 min) over exposure time in terms of gene expression.

In order to establish that our temporal gene expression patterns were caused by heavy metal exposure, and not due to normal physiological changes associated with cell growth, gene expression data of metal-treated *P. fluorescens* cells within each treatment were compared to gene expression data of control cells (i.e. cells grown in the absence of heavy metals). Furthermore, in order to be on the safe side, we selected only those genes whose temporal gene expression response was opposite (overexpression vs. repression) to that found in control cells. On the other hand, for each cluster within each heavy metal treatment, only those genes showing a strong metal-induced molecular response over exposure time were considered: to this aim, we selected those genes whose response at 15 min was ≥2-fold higher (overexpression) or lower (repression) compared to that observed at 5 min.

Gene products were identified with the following databases: *Pseudomonas* Genome Database (Winsor et al. 2011), PseudoCyc (Romero and Karp 2003) and EcoGene 3.0 (Zhou and Rudd 2013). Within each cluster, we selected only those genes with a well-established gene product (i.e. with a known function), not considering in our analysis those genes with a hypothetical or unknown function. These genes were assigned to higher levels of COG (Clusters of Orthologous Groups) functional categories using the http://www-archbac.u-psud.fr/genomics/tree cogs.html website and the *Pseudomonas* Genome Database.

Genes differentially expressed over exposure time under more than one polymetallic treatment and that were not differentially expressed under control treatment were identified as potential biomarkers of the effect of exposure time on the early gene expression of *P. fluorescens* cells exposed to a polymetallic solution.

## Validation of microarray results by RT-qPCR

Reverse transcription quantitative PCR (RT-qPCR) was used to validate our microarray results. Two genes were

randomly selected from each of the abovementioned clusters (repression and overexpression) for all four treatments: then, a total of 16 genes were used for validation. For each gene, the two exposure times were analysed. RT-qPCRs were performed with the same RNA samples used for microarray analysis.

Specific primer pair sets for the 16 selected genes were designed using Primer3 (v.0.4.0) design software (Rozen and Skaletsky 2000). Criteria for primer design were established as in Gómez-Sagasti et al. (2014) (in this case, the size of the amplification product was established between 50 and 200 bp). Primer pair sequences are shown in Table 1.

RNA samples were treated with DNAse I (Invitrogen). cDNA synthesis was done using the High-Capacity cDNA Reverse Transcription Kit (Invitrogen) (Gómez-Sagasti et al. 2014).

Gene expression was measured by qPCR using the qPCR-SYBR Premix ExTaq Perfect Real Time (Takara Bio Inc.) in a 7500 Fast Real-Time PCR System (Applied Biosystems). Briefly, qPCRs were performed in a 25 μL reaction containing 1.5 μL cDNA and 1.5 μL of each primer (final primer concentration=10 μM). Templates were pre-incubated at 50 °C for 2 min, denatured at 95 °C for 10 min and subjected to 40 cycles of the following thermal conditions: 95 °C (15 s) and 55 °C (60 s). For the melt curve, the conditions were 95 °C for 15 s, 60 °C for 1 min and 95 °C for 30 s. Product cycle threshold (Ct) was determined from ROX-normalized fluorescence emission and used to calculate the initial input of the template. RT-qPCR was performed with the two biological replicates of each treatment; in addition, three technical replicates were used for each biological replicate.

RT-qPCRs were analysed using GenEx qPCR analysis software v. 5.4.3 (MultiD Analyses AB). The stability of reference genes was determined using *geNorm* (Vandesompele et al. 2002) and *NormFinder* (Andersen et al. 2004) algorithms integrated in GenEx. Changes in quantification cycle data (ΔCq) of the 16 selected genes were normalized to the more stable reference gene, and then, Cq values were converted to fold expression change values according to the comparative method of Cq (Schmittgen and Livak 2008) with corrected efficiencies (Pfaffl 2006). All statistical analyses were done on $\log_2$-scaled data, except for the correlation analysis between microarray vs. RT-qPCR. Differences in fold expression change values over exposure time for individually analysed genes within each treatment were compared using one-way analysis of variance—ANOVA (LSD post hoc test, $p<0.05$) (SPSS 18.0). Fold-change data for the correlation analysis between microarray vs. RT-qPCR were tested for normality using Shapiro-Wilk test; due to normality, Pearson's correlation coefficient was calculated to determine the level of association between variables (SPSS 18.0).

## Results and discussion

### Effect of polymetallic treatments on *P. fluorescens* growth

At mid-exponential phase, *P. fluorescens* growth was reduced by 4, 20 and 100 % at MD/4, MD and 4MD treatments, respectively, compared to control cells (Fig. 1). When *E. coli* cells were exposed to the same polymetallic treatments (Gómez-Sagasti et al. 2014), they showed a higher sensitivity to heavy metal exposure than our *P. fluorescens* strain. The genus *Pseudomonas* is well-known for its metabolic versatility and tolerance to the presence of organic and inorganic pollutants (Aguilar-Barajas et al. 2010).

### Gene expression in the absence of metals

In the absence of metals (control treatment), 35 genes with a well-defined function were strongly repressed over exposure time (i.e. 35 genes whose response at 15 min was ≥2-fold lower compared to that observed at 5 min) (cluster I, Table 2). Similarly, 28 genes with a well-defined function were strongly overexpressed over exposure time (i.e. 28 genes whose response at 15 min was ≥2-fold higher compared to that observed at 5 min) (cluster II, Table 2).

We observed a repression of genes encoding transport proteins, such as ExbB (iron transport) and GCN5 (*N*-acetyltransferases) (Vetting et al. 2005). On the contrary, genes encoding a glutathione *S*-transferase and a spermidine/putrescine ABC transporter (Igarashi and Kashiwagi 2010) were overexpressed. Genes involved in the regulation of transcription (e.g. AraC, LacI, MerR and Fis families) were also overexpressed. By contrast, other genes involved in transcription regulation (LysR family) were repressed over exposure time.

**Table 1** Primers for RT-qPCR validation of microarray results

| | | | | | |
|---|---|---|---|---|---|
| Reference genes | | | | | |
| *fabD* | Malonyl CoA-ACP transacylase | | F: TCTGCTGGAGCAGCTTTACA/R: GAAAGCGTCTGGGGTATTGA | | (110 bp) |
| *rsd/algQ* | Regulator of RpoD | | F: TCCACGGATCGATGTCATTA/R: TCTTCCTTGTGTGCGGTATG | | (86 bp) |
| *pyr* | Pyrroline-5-carboxylate reductase | | F: TGTAGTGGTACTGGCGGTCA/R: GTTGCTGCTCTTCGTTCAGC | | (149 bp) |
| *16S* | 16S ribosomal RNA | | F: CAAGCGGTGGAGCATGTGG/R: CGACACGAGCTGACGACAG | | (142 bp) |
| Genes for control treatment | | | | | |
| Cluster I (repressed) | | | Cluster II (overexpressed) | | |
| *lysR* | F: GAGTGAATCGCGGATCTGC | 150 bp | *DEAD* | F: TGCGGGAAGAACTGGATATT | 155 bp |
| Transcriptional regulator 3 | R: GCACAGGCCTGATCAAACG | | DEAD/DEAH box helicase-like | R: GGCAAGTTT TTCCGAACTCA | |
| *mo-FAD* | F: AATGTCGAAACCAGCCTGAC | 129 bp | *Zn-Alcohol* | F: TCC CGC TGG ATACTTTCATC | 101 bp |
| Molybdopterin DHase, FAD-binding | R: GACGGACACTAGAGCGAAGG | | Zn-containing alcohol DHse | R: ATGAATGACGGTACGGATGC | |
| Genes for MD/4 treatment | | | | | |
| Cluster I (repressed) | | | Cluster II (overexpressed) | | |
| *F3* | F: CGCTTGCAGTACCAGATCCC | 132 bp | *chaC* | F: CGACACCTGCCCAGCTATG | 148 bp |
| Extracellular solute-binding protein 3 | R: CTGCTCCAGACGGCCATC | | ChaC-like protein | R: GATTCCGGTCTGGCATGGC | |
| *hsp70* | F: GCCAAGGTTGAAGAGCTGTC | 159 bp | *GS* | F: CGCCTACGACCACGAGATG | 140 bp |
| Heat shock protein Hsp70 | R: CTTGTGGTCTTTGACTTCTTCG | | L-glutamine synthetase | R: CGTTCTGCTCGGGATCCTC | |
| Genes for MD treatment | | | | | |
| Cluster I (repressed) | | | Cluster II (overexpressed) | | |
| *tonB* | F: GCACCTCGCGTACCAACTAC | 113 bp | *chem* | F: GGCCGAGGAGCAGAGTTC | 148 bp |
| TonB-dependent receptor | R: GTTGGTGACCGAGCCGTAG | | Chemotaxis sensory transducer | R: CATCCCCCGCAACTCACTC | |
| *cytC* | F: AACCAAGCTCAAGACCATGC | 145 bp | *GCN5* | F: GCTATTGAGCGGGGCGAAC | 132 bp |
| Cyt oxidase, mono-heme subunit | R: CTTGCTTTTGATCGCAGTGC | | GCN5-related *N*-acetyltransferase | R: CCGGCCCTGAAAGTGTTGG | |
| Genes for 4MD treatment | | | | | |
| Cluster I (repressed) | | | Cluster II (overexpressed) | | |
| *ATPase* | F: CGTCATACCGCCGCTATCC | 141 bp | *met* | F: CACGATCAAGGAGGGTGAGC | 117 bp |
| HM-translocating P-type ATPase | R: CACCACCAGCAGGCTCAC | | Metallothionein (P) | R: CGCACACTGACACCCCTTG | |
| *PFKB* | F: CCGACCCGTTCCGAACTAG | 131 bp | *oxo* | F: GGGGCTGGAAGCGATCTTC | 143 bp |
| Phosphofructokinase | R: GGCCTCATCGCTGGCAAG | | 3-oxoacyl-ACP synthase II | R: ACGCATACTCGATCGGCATC | |

*ACP* acyl carrier protein; *DHase* dehydrogenase; *Cyt* cytochrome; *HM* heavy metal; *MD* (metal dose)=10 μM Ag(I), 10 μM Pb(II), 10 μM Cd(II), 10 μM Cu(II), 500 μM Ni(II) and 300 μM Zn(II); *MD/4* fourfold lower metal dose; *4MD* fourfold higher metal dose; *F* forward; *R* reverse

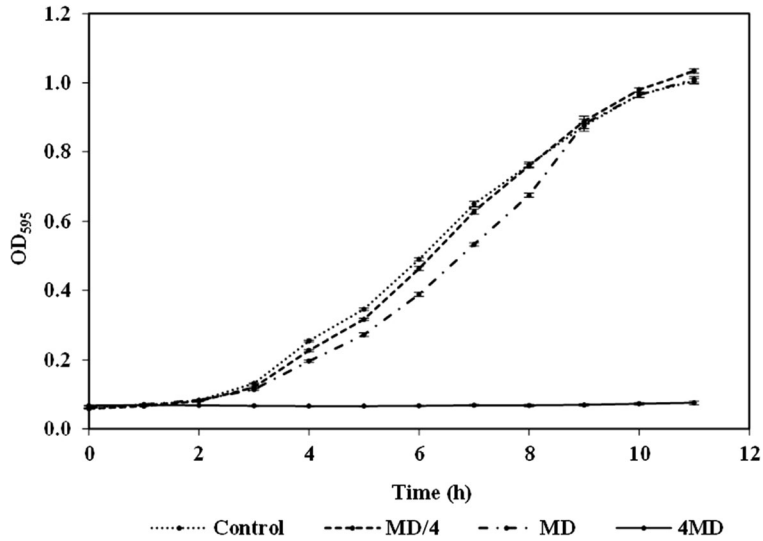


**Fig. 1** Effect of polymetallic treatments on *P. fluorescens* cell growth. *MD* (metal dose)=10 μM Ag(I), 10 μM Pb(II), 10 μM Cd(II), 10 μM Cu(II), 500 μM Ni(II) and 300 μM Zn(II). *MD/4* fourfold lower metal dose, *4MD* fourfold higher metal dose. *Bars*, standard deviations ($n$=4)

Gene expression at the lowest metal dose (MD/4 treatment)

Under MD/4 treatment, 51 and 55 genes were strongly repressed (cluster I) and overexpressed (cluster II), respectively (Table 3). Owing to the large number of genes included in Table 3 (also in Tables 4 and 5), here and elsewhere in this paper, only those genes involved in (i) heavy metal-related processes, (ii) oxidative stress responses or (iii) relevant cellular functions under stressing conditions are discussed.

Concerning stimuli signalling, we observed the repression (cluster I) of a gene encoding a TonB-dependent copper receptor (Table 3). According to Hu et al. (2005), this type of receptor can be used as sensor of external stimuli (e.g. presence of heavy metals) and, unlike here in this study, is usually overexpressed in the presence of the stimulus. Two genes encoding sensor histidine kinase proteins located in the periplasm (Mascher et al. 2006; Krell et al. 2010) were also repressed under MD/4 treatment.

We observed the repression of genes encoding ABC membrane transporters involved in multidrug resistance (MDR) pumps, such as MatE and MdtK which belong to the MATE-family transporters for multidrug and toxic compound extrusion (Omote et al. 2006), and also the repression of genes encoding transporters from the major facilitator superfamily (MFS) (Lubelski et al. 2007; Kumar et al. 2013). MDR pumps are capable of extruding heavy metals (Silver and Phung 2005; Martínez et al. 2009). Reva et al. (2006) and Pagès et al. (2007) found a metal-induced overexpression of MFS genes in *Pseudomonas putida* and *Pseudomonas brassicacearum*, respectively.

MD/4 treatment led to a repression of *hsp70* (*dnaK*), *dnaJ* and *grpE* genes (Table 3). In *E. coli* cells, these three genes are implicated in heat shock responses (Dubern et al. 2005). Sharma et al. (2008) also observed the inhibitory effect of $Cd^{2+}$, $Hg^{2+}$ and $Pb^{2+}$ on Hsp70-assisted (DnaK/DnaJ/GrpE) protein folding. In addition, the *clpA* gene encoding an ATP-dependent Clp protease was also repressed under MD/4 treatment. Li et al. (2012) found that *clp* gene rupture results in an activation of chaperone expression and, in turn, an inhibition of enzymes related to tRNA modification.

Pertaining to cluster II (Table 3), we observed the overexpression of genes involved in (i) transport (ChaC: $Ca^{2+}/H^{+}$ antiporter putatively associated with cobalt transport; CopC: copper resistance; ABC transporter), (ii) entry of drugs and toxins (outer membrane porin proteins) and (iii) chemotaxis (EnvZ). Our analysis revealed the overexpression of two genes encoding glutamine synthetase (GS), an enzyme involved in nitrogen metabolism (Forchhammer 2007), but the role of this GS overexpression in response to the presence of metals is not clear. Pagès et al. (2007) reported a GS overexpression in Cd-exposed *P. brasicacearum* cells. In *E. coli*, we also observed

**Table 2** *P. fluorescens* genes differentially ($p<0.01$) expressed over exposure time in the absence of metals (control cells)

| COG class and gene description | Probe name | FC |
|---|---|---|
| Cluster I (repressed): 35 genes | | |
| Cellular processes and signalling (34 %) | | |
| Cell wall/membrane/envelope biogenesis | | |
| UDP-glucose 6-dehydrogenase | CUST_6384_PI425702210 | 3.5 |
| General substrate transporter | CUST_2898_PI425702210 | 2.7 |
| Insecticidal toxin protein (P) | CUST_946_PI425702210 | 2.1 |
| Defence mechanisms | | |
| Beta-lactamase-like protein | CUST_1448_PI425702210 | 2.2 |
| Intracellular trafficking, secretion and vesicular transport | | |
| General secretion pathway M protein | CUST_3160_PI425702210 | 2.8 |
| ExbB/TonB protein, uptake of enterochelin/B colicins (P) | CUST_6908_PI425702210 | 2.4 |
| MotA/TolQ/ExbB proton channel | CUST_5531_PI425702210 | 2.3 |
| Heat shock protein (Hsp20) | CUST_1878_PI425702210 | 2.3 |
| Peptidyl-prolyl *cis-trans* isomerase, cyclophilin type | CUST_1423_PI425702210 | 2.0 |
| Signal transduction mechanisms | | |
| Periplasmic sensor signal transduction histidine kinase | CUST_2821_PI425702210 | 4.1 |
| EAL domain protein | CUST_48_PI425702210 | 3.9 |
| Diguanylate cyclase (GGDEF domain) (P) | CUST_4062_PI425702210 | 2.7 |
| Information storage and processing (26 %) | | |
| Post-translational modification, protein turnover, chaperones | | |
| ATP-dependent protease (HslV) | CUST_5882_PI425702210 | 2.2 |
| Replication, recombination and repair | | |
| DEAD_2 | CUST_2679_PI425702210 | 7.3 |
| Transcription | | |
| Transcriptional regulator, LysR family | CUST_3665_PI425702210 | 3.1 |
| Transcriptional regulator, LysR family | CUST_2409_PI425702210 | 2.9 |
| Aminotransferase, class I and II | CUST_1708_PI425702210 | 2.9 |
| Sigma-32 (RpoH) | CUST_5312_PI425702210 | 2.2 |
| Nucleoside diphosphate pyrophosphatase | CUST_493_PI425702210 | 2.1 |
| Translation, ribosomal structure and biogenesis | | |
| GCN5-related *N*-acetyltransferase | CUST_2545_PI425702210 | 2.2 |
| GCN5-related *N*-acetyltransferase | CUST_1905_PI425702210 | 2.0 |
| Metabolism (29 %) | | |
| Amino acid transport and metabolism | | |
| Glyoxalase family protein | CUST_6219_PI425702210 | 3.3 |
| Coenzyme transport and metabolism | | |
| Molybdopterin dehydrogenase, FAD-binding | CUST_2102_PI425702210 | 4.4 |
| Thiamine pyrophosphate enzyme | CUST_4449_PI425702210 | 2.1 |
| Cobalamin biosynthesis protein | CUST_5796_PI425702210 | 2.1 |
| Ferrochelatase | CUST_4708_PI425702210 | 2.0 |
| Energy production and conversion | | |
| Aldehyde dehydrogenase | CUST_3260_PI425702210 | 4.1 |
| Iron-sulfur cluster-binding protein | CUST_3258_PI425702210 | 3.9 |
| Electron transport complex protein (RnfA) | CUST_4486_PI425702210 | 2.1 |

**Table 2** (continued)

| COG class and gene description | Probe name | FC |
|---|---|---|
| Inorganic ion transport and metabolism | | |
| Arsenate reductase | CUST_4206_PI425702210 | 3.1 |
| 3 (2),5-bisphosphate nucleotidase (CysQ) | CUST_5859_PI425702210 | 2.6 |
| Poorly characterized (9 %) | | |
| Function unknown | | |
| Membrane protein (P) | CUST_6337_PI425702210 | 4.1 |
| Fusaric acid resistance protein FusA precursor | CUST_6553_PI425702210 | 2.0 |
| General function prediction only | | |
| ThiJ/PfpI family protein | CUST_2221_PI425702210 | 2.2 |
| Unclassified (2 %) | | |
| Kelch repeat-containing protein | CUST_2516_PI425702210 | 2.5 |
| Cluster II (overexpressed): 28 genes | | |
| Cellular processes and signalling (43 %) | | |
| Cell wall/membrane/envelope biogenesis | | |
| Outer membrane porin | CUST_2621_PI425702210 | 2.6 |
| Defence mechanisms | | |
| Secretion protein (HlyD) | CUST_229_PI425702210 | 2.2 |
| Intracellular trafficking, secretion and vesicular transport | | |
| Flp/Fap pilin component | CUST_648_PI425702210 | 2.7 |
| Post-translational modification, protein turnover, chaperones | | |
| DnaJ-domain containing protein | CUST_6844_PI425702210 | 2.7 |
| Protoheme IX farnesyltransferase | CUST_4622_PI425702210 | 2.3 |
| 2OG-Fe(II) oxygenase | CUST_2325_PI425702210 | 2.1 |
| Glutathione *S*-transferase family protein | CUST_6569_PI425702210 | 2.0 |
| PII uridylyl-transferase (GlnB) | CUST_1096_PI425702210 | 2.0 |
| Signal transduction mechanisms | | |
| HTH-type transcriptional regulator (GltR) | CUST_6480_PI425702210 | 3.1 |
| Possible transcriptional regulator, Fis family | CUST_2964_PI425702210 | 2.6 |
| Response regulator receiver domain protein (CheY) | CUST_2639_PI425702210 | 2.3 |
| Sigma-E regulatory protein, MucB/RseB | CUST_1359_PI425702210 | 2.3 |
| Information storage and processing (21 %) | | |
| Replication, recombination and repair | | |
| DEAD/DEAH box helicase-like protein | CUST_1202_PI425702210 | 4.1 |
| Transcriptional regulator, AraC family | CUST_2454_PI425702210 | 2.6 |
| Transcription | | |
| Transcriptional regulator, LacI family | CUST_247_PI425702210 | 3.1 |
| Transcriptional regulator, MerR family | CUST_658_PI425702210 | 2.1 |
| Ribonuclease inhibitor barstar | CUST_2033_PI425702210 | 2.0 |
| Translation, ribosomal structure and biogenesis | | |
| Translation elongation factor 2 (EF-2/EF-G) | CUST_5060_PI425702210 | 2.4 |
| Metabolism (21 %) | | |
| Amino acid transport and metabolism | | |
| Spermidine/putrescine ABC transporter ATP-binding subunit | CUST_5103_PI425702210 | 2.1 |

**Table 2** (continued)

| COG class and gene description | Probe name | FC |
|---|---|---|
| Coenzyme transport and metabolism | | |
| Molybdenum cofactor synthesis-like protein | CUST_4125_PI425702210 | 2.6 |
| Energy production and conversion | | |
| Isocitrate lyase | CUST_3585_PI425702210 | 2.3 |
| NADH dehydrogenase (ubiquinone), subunit | CUST_3589_PI425702210 | 2.3 |
| NADH-quinone oxidoreductase, chain I | CUST_3593_PI425702210 | 2.2 |
| Secondary metabolites biosynthesis, transport and catabolism | | |
| Dienelactone hydrolase | CUST_4220_PI425702210 | 2.1 |
| Poorly characterized (11 %) | | |
| General function prediction only | | |
| Zinc-containing alcohol dehydrogenase superfamily | CUST_1122_PI425702210 | 5.7 |
| ATPase associated with various cellular activities, AAA_3 | CUST_4421_PI425702210 | 5.1 |
| ABC transporter-like protein | CUST_554_PI425702210 | 2.3 |
| Unclassified (4 %) | | |
| Dimethylmenaquinone methyltransferase | CUST_2973_PI425702210 | 2.0 |

*FC* fold change between 5 and 15 min exposure time, *P* putative

the overexpression of GS under MD/4 and MD treatments (Gómez-Sagasti et al. 2014).

## Gene expression at the intermediate metal dose (MD treatment)

Under MD treatment, 55 and 72 genes were strongly repressed (cluster I) and overexpressed (cluster II), respectively (Table 4). Genes involved in amino acid metabolism (e.g. arginine/ornithine antiporter, glutamine amidotransferase class I, glutamate dehydrogenase) were repressed. Like in the MD/4 treatment, a gene encoding a TonB-dependent receptor and two genes encoding a PAS/PAC sensor signal transduction histidine kinase located in the periplasm were also repressed. We also observed the repression of genes encoding MscS proteins (mechanosensitive channels), CheY protein involved in signalling and reception of stimuli, DNA topoisomerase and helicase and NUDIX hydrolase (MutT: repair of oxidative damage) (Braz and Marques 2005). In a previous work (Gómez-Sagasti et al. 2014), in *E. coli* cells, we observed the repression of *mutY* gene which functions synergistically with *mutT* to protect the cell from deleterious effects on DNA; then, its repression might be understood as an early symptom of metal toxicity. On the contrary, the *mutT* gene was overexpressed in metal-exposed *Caulobacter crescentus* cells (Hu et al. 2005). Finally, several families of transcription regulators (HxlR, AraC, LysR,) were repressed under this intermediate metal treatment.

Regarding cluster II (overexpression), we observed the overexpression of genes encoding the Sigma-24 factor (FecI protein) and FecR protein for the uptake and transport of ferric chelates (Potvin et al. 2007; Llamas and Bitter 2010; Saha et al. 2013). Likewise, genes encoding signalling proteins such as a heavy metal sensor histidine kinase and methyl-accepting chemotaxis proteins (MCP), which are usually methylated by protein methyltransferase CheR (Bi and Lai 2014), were overexpressed. Interestingly, sulfur metabolism was stimulated by MD treatment, as reflected by the overexpression of a sulfate permease, a sulfatase, a thiol:disulfide interchange protein and oxidorreductase (DsbE/CcmG), a sulfate/thiosulfate-binding protein and three genes encoding taurine ABC transporters (taurine is a sulfur-containing amino acid). We observed a similar response in *E. coli* cells exposed to MD/4 and MD treatments for some genes involved in the biosynthesis of cysteine (another sulfur-rich amino acid) (Gómez-Sagasti et al. 2014). In *E. coli*, it has been postulated that the Dsb system (e.g. DsbE) can repair non-native disulfide bonds in the periplasm (Collet and Bardwell 2002). Teitzel et al. (2006) also observed the induction of a thiol:disulfide interchange protein and taurine ABC permeases in Cu-exposed *Pseudomonas aeruginosa*.

**Table 3** *P. fluorescens* genes differentially ($p<0.01$) expressed over exposure time under MD/4 metal treatment

| COG class and gene description | Probe name | FC |
|---|---|---|
| Cluster I (repressed): 51 genes | | |
| Cellular processes and signalling (39 %) | | |
| Cell cycle control, cell division, chromosome partitioning | | |
| ATPase domain-containing protein | CUST_3174_PI425702210 | 2.8 |
| Cell motility/intracellular trafficking, secretion and vesicular transport | | |
| Chemotaxis phosphatase (CheZ) | CUST_1560_PI425702210 | 2.3 |
| Flagellar motor switch protein (FliG) | CUST_1532_PI425702210 | 2.3 |
| Flagellar assembly protein (FliH) | CUST_1533_PI425702210 | 2.1 |
| Cell wall/membrane/envelope biogenesis | | |
| Lipopolysaccharide biosynthesis-associated protein | CUST_2803_PI425702210 | 3.5 |
| Insecticidal toxin protein (P) | CUST_946_PI425702210[a] | 2.6 |
| UDP-glucose pyrophosphorylase | CUST_3814_PI425702210 | 2.6 |
| Penicillin-binding protein 1A | CUST_402_PI425702210 | 2.2 |
| UDP-glucose pyrophosphorylase | CUST_2917_PI425702210 | 2.0 |
| Defence mechanisms | | |
| Multi antimicrobial extrusion protein (MatE) | CUST_3524_PI425702210 | 3.1 |
| Multidrug resistance protein (MdtK) | CUST_6606_PI425702210 | 2.5 |
| Post-translational modification, protein turnover, chaperones | | |
| ATP-dependent Clp protease ATP-binding subunit (ClpA) | CUST_3573_PI425702210 | 3.2 |
| PIM1 peptidase. Serine peptidase. MEROPS family S16 | CUST_4557_PI425702210 | 3.1 |
| Heat shock protein (Hsp70) | CUST_760_PI425702210 | 2.5 |
| Urease accessory protein (UreG) | CUST_559_PI425702210 | 2.4 |
| GrpE protein | CUST_759_PI425702210 | 2.2 |
| Chaperone protein (DnaJ) | CUST_5947_PI425702210 | 2.1 |
| Signal transduction mechanisms | | |
| Isocitrate dehydrogenase kinase/phosphatase | CUST_1414_PI425702210 | 3.1 |
| Periplasmic sensor signal transduction histidine kinase | CUST_1998_PI425702210 | 2.5 |
| Periplasmic sensor signal transduction histidine kinase | CUST_2821_PI425702210[a] | 2.1 |
| Information storage and processing (8 %) | | |
| Transcription | | |
| Transcriptional regulator, LysR family | CUST_2714_PI425702210 | 3.4 |
| Transcriptional regulator, AraC family | CUST_2815_PI425702210 | 2.7 |
| Translation, ribosomal structure and biogenesis | | |
| GCN5-related *N*-acetyltransferase | CUST_2181_PI425702210 | 2.4 |
| GCN5-related *N*-acetyltransferase | CUST_3349_PI425702210 | 2.2 |
| Metabolism (33 %) | | |
| Amino acid transport and metabolism | | |
| Extracellular solute-binding protein, family 3 | CUST_3289_PI425702210 | 3.0 |
| Aminotransferase, class I and II | CUST_1708_PI425702210[a] | 2.3 |
| Fumarate reductase/succinate dehydrogenase flavoprotein-like | CUST_4889_PI425702210 | 2.1 |
| Arginine/ornithine antiporter | CUST_4362_PI425702210 | 2.1 |
| GABA permease | CUST_2284_PI425702210 | 2.0 |
| Carbohydrate transport and metabolism | | |
| Phosphoenolpyruvate synthase | CUST_1765_PI425702210 | 2.9 |

**Table 3** (continued)

| COG class and gene description | Probe name | FC |
|---|---|---|
| Major facilitator superfamily MFS 1 | CUST_657_PI425702210 | 2.4 |
| Xylose isomerase-like TIM barrel | CUST_4887_PI425702210 | 2.3 |
| Shikimate dehydrogenase | CUST_3696_PI425702210 | 2.1 |
| Energy production and conversion | | |
| Cytochrome c, class I | CUST_2728_PI425702210 | 2.5 |
| Inorganic ion transport and metabolism | | |
| 3 (2),5-bisphosphate nucleotidase (CysQ) | CUST_5859_PI425702210[a] | 2.2 |
| TonB-dependent copper receptor | CUST_5926_PI425702210 | 2.0 |
| Lipid transport and metabolism | | |
| Acyltransferase 3 | CUST_2599_PI425702210 | 2.1 |
| Acyltransferase 3 | CUST_5431_PI425702210 | 2.0 |
| Nucleotide transport and metabolism | | |
| Hydroxydechloroatrazine ethylaminohydrolase | CUST_3418_PI425702210 | 2.4 |
| Secondary metabolites biosynthesis, transport and catabolism | | |
| *N*-Hydroxyarylamine *O*-acetyltransferase | CUST_6146_PI425702210 | 2.0 |
| Aromatic-ring hydroxylase | CUST_2967_PI425702210 | 2.0 |
| Poorly characterized (8 %) | | |
| Function unknown | | |
| UDP-2,3-diacylglucosamine hydrolase | CUST_3624_PI425702210 | 2.3 |
| General function prediction only | | |
| Virulence factor MVIN-like | CUST_4832_PI425702210 | 4.6 |
| Metal dependent phosphohydrolase, HD region | CUST_3954_PI425702210 | 2.5 |
| Peptidase C56 (PfpI) | CUST_1300_PI425702210 | 2.4 |
| Unclassified (12 %) | | |
| Nuclease (SNase-like) | CUST_400_PI425702210 | 4.0 |
| $H^+$-transporting two-sector ATPase, delta/epsilon subunit | CUST_5700_PI425702210 | 3.5 |
| Rhizobiocin (RzcA) | CUST_6686_PI425702210 | 3.2 |
| L-Seryl-tRNA (Sec) selenium transferase | CUST_2695_PI425702210 | 2.8 |
| IndB protein | CUST_6665_PI425702210 | 2.2 |
| Probable bacteriophage signal peptide protein | CUST_1170_PI425702210 | 2.1 |
| Cluster II (overexpressed): 55 genes | | |
| Cellular processes and signalling (31 %) | | |
| Cell cycle control, cell division, chromosome partitioning | | |
| Filamentation induced by cAMP protein Fic | CUST_4982_PI425702210 | 2.0 |
| Cell wall/membrane/envelope biogenesis | | |
| Outer membrane porin | CUST_1274_PI425702210 | 3.0 |
| Membrane bound *O*-acyl transferase, MBOAT | CUST_950_PI425702210 | 2.0 |
| Defence mechanisms | | |
| Type I secretion membrane fusion protein (HlyD) | CUST_2670_PI425702210 | 3.0 |
| Secretion protein (HlyD) | CUST_4470_PI425702210 | 2.5 |
| Post-translational modification, protein turnover, chaperones | | |
| Protoheme IX farnesyltransferase | CUST_4622_PI425702210[a] | 2.5 |
| DSBA oxidoreductase | CUST_52_PI425702210 | 2.5 |

**Table 3** (continued)

| COG class and gene description | Probe name | FC |
|---|---|---|
| Replication, recombination and repair | | |
| SMF protein | CUST_19_PI425702210 | 2.1 |
| Signal transduction mechanisms | | |
| Osmolarity sensor protein (EnvZ) | CUST_259_PI425702210 | 3.9 |
| Chemotaxis sensory transducer | CUST_5491_PI425702210 | 3.0 |
| Histidine kinase | CUST_286_PI425702210 | 2.1 |
| Transcription | | |
| Transcriptional regulator, GntR family | CUST_1788_PI425702210 | 2.9 |
| Two component transcriptional regulator, winged helix family | CUST_3913_PI425702210 | 2.3 |
| Transcriptional regulator, AraC family | CUST_2454_PI425702210[a] | 2.1 |
| Translation, ribosomal structure and biogenesis | | |
| GCN5-related *N*-acetyltransferase | CUST_2605_PI425702210 | 3.1 |
| Acetyltransferase, GNAT family | CUST_3631_PI425702210 | 2.8 |
| RNA-binding S4 | CUST_1186_PI425702210 | 2.1 |
| Metabolism (46 %) | | |
| Amino acid transport and metabolism | | |
| Periplasmic binding protein | CUST_4987_PI425702210 | 3.5 |
| Shikimate 5-dehydrogenase | CUST_6809_PI425702210 | 3.4 |
| Lysine exporter protein (LYSE/YGGA) | CUST_1705_PI425702210 | 3.3 |
| Amino acid permease-associated region | CUST_4012_PI425702210 | 2.8 |
| Extracellular solute-binding protein, family 1 | CUST_5102_PI425702210 | 2.7 |
| Hydroxymethylglutaryl-CoA lyase | CUST_1312_PI425702210 | 2.7 |
| Extracellular solute-binding protein, family 1 | CUST_2817_PI425702210 | 2.4 |
| L-Glutamine synthetase | CUST_2116_PI425702210 | 2.3 |
| Glutamine synthetase (P) | CUST_6881_PI425702210 | 2.1 |
| Carbohydrate transport and metabolism | | |
| Glycerol-3-phosphate transporter | CUST_5427_PI425702210 | 3.8 |
| Xylose isomerase-like TIM barrel | CUST_2889_PI425702210 | 2.7 |
| Gluconolactonase | CUST_3437_PI425702210 | 2.6 |
| Polysaccharide deacetylase | CUST_2832_PI425702210 | 2.3 |
| Coenzyme transport and metabolism | | |
| Cobyrinic acid a,c-diamide synthase | CUST_440_PI425702210 | 2.1 |
| Energy production and conversion | | |
| Malate synthase G | CUST_5161_PI425702210 | 2.0 |
| Cytochrome bd ubiquinol oxidase, subunit I | CUST_4871_PI425702210 | 2.0 |
| Inorganic ion transport and metabolism | | |
| ChaC-like protein | CUST_5428_PI425702210 | 3.3 |
| Thiosulfate sulfurtransferase | CUST_5116_PI425702210 | 2.4 |
| Lipid transport and metabolism | | |
| 3-Hydroxyisobutyrate dehydrogenase | CUST_694_PI425702210 | 3.4 |
| Glucose 1-dehydrogenase | CUST_1835_PI425702210 | 2.4 |
| Short-chain dehydrogenase/reductase SDR | CUST_2459_PI425702210 | 2.1 |
| Short-chain dehydrogenase/reductase SDR | CUST_3403_PI425702210 | 2.1 |

**Table 3** (continued)

| COG class and gene description | Probe name | FC |
|---|---|---|
| Nucleotide transport and metabolism | | |
| Xanthine dehydrogenase, molybdenum binding subunit apoprotein | CUST_2101_PI425702210 | 2.4 |
| Phosphoribosylaminoimidazole carboxylase | CUST_5597_PI425702210 | 2.2 |
| Secondary metabolites biosynthesis, transport and catabolism | | |
| Copper resistance protein (CopC) | CUST_3430_PI425702210 | 2.3 |
| Poorly characterized (18 %) | | |
| Function unknown | | |
| Aromatic-ring-hydroxylating dioxygenase, beta-subunit | CUST_2955_PI425702210 | 2.6 |
| YceI-like family protein | CUST_5262_PI425702210 | 2.5 |
| Membrane protein (P) | CUST_5933_PI425702210 | 2.3 |
| Membrane protein (P) | CUST_6820_PI425702210 | 2.0 |
| General function prediction only | | |
| Esterase (P) | CUST_1123_PI425702210 | 8.1 |
| Zinc-containing alcohol dehydrogenase superfamily | CUST_1122_PI425702210[a] | 7.3 |
| ABC transporter-like protein | CUST_554_PI425702210[a] | 3.0 |
| NADPH-dependent FMN reductase | CUST_2184_PI425702210 | 2.8 |
| TRNA modification GTPase (TrmE) | CUST_5713_PI425702210 | 2.3 |
| Alpha/beta hydrolase fold family | CUST_3917_PI425702210 | 2.0 |
| Unclassified (5 %) | | |
| Major royal jelly protein | CUST_2703_PI425702210 | 2.1 |
| Lipopolysaccharide kinase | CUST_468_PI425702210 | 2.1 |
| P-loop ATPase protein UPF0042 (P) | CUST_851_PI425702210 | 2.1 |

*FC* fold change between 5 and 15 min exposure time, *P* putative

[a] Genes that were also differentially expressed over exposure time in the absence of metals

These genes could be important for cell growth in the presence of an excess of heavy metals, since some cations, particularly "sulfur lovers", can be segregated into complex compounds by thiol-containing molecules (Nies 2003).

Similarly, genes involved in oxidative stress (e.g. 1-Cys peroxiredoxin and alkyl hydroperoxide reductase subunit), alginate biosynthesis (e.g. AlgJ), cell wall peptidase (NlpC/P60 family) (Anantharamn and Aravind 2003), and active transport systems (ExbD/TolR and MotA/TolQ/ExbB for siderophore-chelated iron) (Ma et al. 2009; Schalk et al. 2011) were overexpressed. *E. coli* cells under MD/4 and MD treatments exhibited the overexpression of *exbB* and alkyl hydroperoxide reductase, respectively (Gómez-Sagasti et al. 2014), suggesting their involvement in a possible protection mechanism against metal stress.

Finally, we found an overexpression of transcription regulators (e.g. DeoR, GntR and IclR), a RpoH heat shock transcription factor (Sigma-32) (Potvin et al. 2007) and a gene encoding the LexA transcriptional repressor (a key component of the SOS response) (Butala et al. 2009).

## Gene expression at the highest metal dose (4MD treatment)

Under 4MD treatment, 356 and 375 genes were strongly repressed (cluster I) and overexpressed (cluster II), respectively (Table 5). Interestingly, some genes related to heavy metal and metalloid transport were repressed: an arsenical pump, the cobalt transporter subunit CbtA, a heavy metal-(Cd/Co/Hg/Pb/Zn)-P-type ATPase and FecR and FecI (sigma-24) genes. Moreover, we

**Table 4** *P. fluorescens* genes differentially ($p<0.01$) expressed over exposure time under MD metal treatment

| COG class and gene description | Probe name | FC |
|---|---|---|
| Cluster I (repressed): 55 genes | | |
| Cellular processes and signalling (16 %) | | |
| Cell motility | | |
| Flagellar basal body rod protein | CUST_1496_PI425702210 | 2.0 |
| Intracellular trafficking, secretion and vesicular transport | | |
| Hemolysin-type $Ca^{2+}$-binding repeat protein | CUST_6070_PI425702210 | 4.1 |
| Post-translational modification, protein turnover, chaperones | | |
| Cytochrome c oxidase cbb3-type, subunit I | CUST_1822_PI425702210 | 2.6 |
| Signal transduction mechanisms | | |
| Response regulator receiver modulated diguanylate cyclase/phosphodiesterase | CUST_4530_PI425702210 | 3.3 |
| Response regulator receiver domain protein (CheY) | CUST_5719_PI425702210 | 2.8 |
| Response regulator receiver domain protein (CheY) | CUST_4528_PI425702210 | 2.4 |
| PAS/PAC sensor signal transduction histidine kinase | CUST_2242_PI425702210 | 2.3 |
| PAS/PAC sensor signal transduction histidine kinase | CUST_1528_PI425702210 | 2.1 |
| MscS mechanosensitive ion channel | CUST_4398_PI425702210 | 2.1 |
| Information storage and processing (27 %) | | |
| Replication, recombination and repair | | |
| DNA topoisomerase I | CUST_3854_PI425702210 | 2.8 |
| Replication restart DNA helicase (PriA) | CUST_398_PI425702210 | 2.6 |
| Transcription | | |
| Transcriptional regulator, HxlR family | CUST_3255_PI425702210 | 4.4 |
| Transcriptional regulator Ada/DNA-O6-methylguanine-protein-Cys-S-methyltransferase | CUST_2150_PI425702210 | 4.1 |
| Transcriptional regulator, AraC family | CUST_2250_PI425702210 | 3.0 |
| Transcriptional regulator, AraC family with amidase-like domain | CUST_5207_PI425702210 | 2.6 |
| Probable transcription regulator protein | CUST_6032_PI425702210 | 2.3 |
| Two component heavy metal response transcriptional regulator, winged helix family | CUST_3833_PI425702210 | 2.1 |
| Two component, Sigma-54 specific, transcriptional regulator, Fis family | CUST_1999_PI425702210 | 2.1 |
| Transcriptional regulator, MarR family | CUST_2757_PI425702210 | 2.0 |
| Transcriptional regulator, LysR family | CUST_3266_PI425702210 | 2.0 |
| RNA binding S1 | CUST_257_PI425702210 | 2.0 |
| Translation, ribosomal structure and biogenesis | | |
| Heat shock protein (Hsp20) | CUST_1878_PI425702210* | 3.0 |
| Endoribonuclease L-PSP | CUST_3072_PI425702210 | 2.2 |
| LSU ribosomal protein L32P | CUST_4138_PI425702210 | 2.0 |
| Metabolism (42 %) | | |
| Amino acid transport and metabolism | | |
| Substrate-binding region of ABC-type glycinebetaine transport system | CUST_5216_PI425702210 | 3.6 |
| Arginine/ornithine antiporter | CUST_6714_PI425702210 | 2.5 |
| Glutamate dehydrogenase (NAD) | CUST_3190_PI425702210 | 2.4 |
| Aminotransferase | CUST_288_PI425702210 | 2.3 |
| Arginine/ornithine antiporter | CUST_4363_PI425702210 | 2.2 |
| Branched-chain alpha-keto acid dehydrogenase E2 component | CUST_3449_PI425702210 | 2.1 |
| ACT domain protein | CUST_5160_PI425702210 | 2.0 |
| Arginine/ornithine antiporter | CUST_4362_PI425702210 | 2.0 |

**Table 4** (continued)

| COG class and gene description | Probe name | FC |
|---|---|---|
| Carbamate kinase | CUST_4366_PI425702210 | 2.0 |
| 4-Hydroxyphenylpyruvate dioxygenase | CUST_2905_PI425702210 | 2.0 |
| Carbohydrate transport and metabolism | | |
| Transketolase subunit B | CUST_2707_PI425702210 | 3.6 |
| Membrane protein involved in the export of O-antigen and teichoic acid-like | CUST_2009_PI425702210 | 3.0 |
| Coenzyme transport and metabolism | | |
| Dihydroneopterin aldolase family | CUST_5122_PI425702210 | 3.7 |
| Energy production and conversion | | |
| Cytochrome C oxidase, mono-heme subunit/FixO | CUST_1821_PI425702210 | 2.8 |
| Inorganic ion transport and metabolism | | |
| Sulfatase | CUST_2573_PI425702210 | 3.8 |
| TonB-dependent receptor | CUST_3108_PI425702210 | 3.1 |
| Lipid transport and metabolism | | |
| Lipolytic enzyme, G-D-S-L | CUST_3992_PI425702210 | 2.5 |
| Short-chain dehydrogenase/reductase SDR | CUST_4084_PI425702210 | 2.5 |
| Short-chain dehydrogenase/reductase SDR | CUST_4172_PI425702210 | 2.3 |
| Short-chain dehydrogenase/reductase SDR | CUST_2857_PI425702210 | 2.0 |
| Nucleotide transport and metabolism | | |
| NUDIX hydrolase | CUST_3274_PI425702210 | 5.0 |
| Secondary metabolites biosynthesis, transport and catabolism | | |
| Thioesterase superfamily | CUST_1021_PI425702210 | 2.3 |
| Fumarylacetoacetate hydrolase | CUST_908_PI425702210 | 2.2 |
| Poorly characterized (9 %) | | |
| General function prediction only | | |
| Phospholipase/carboxylesterase | CUST_2060_PI425702210 | 4.0 |
| Auxin efflux carrier | CUST_828_PI425702210 | 2.8 |
| Glutamine amidotransferase class I | CUST_5489_PI425702210 | 2.7 |
| FAD-dependent pyridine nucleotide-disulfideoxidoreductase | CUST_3041_PI425702210 | 2.6 |
| Helicase (P) | CUST_6344_PI425702210 | 2.1 |
| Unclassified (6 %) | | |
| Amino acid efflux transmembrane protein (P) | CUST_6046_PI425702210 | 2.7 |
| MGC80314 protein (P) | CUST_6448_PI425702210 | 2.6 |
| IndB protein | CUST_6665_PI425702210 | 2.5 |
| Cluster II (overexpressed): 72 genes | | |
| Cellular processes and signalling (28 %) | | |
| Cell motility | | |
| Methyl-accepting chemotaxis protein | CUST_6674_PI425702210 | 7.9 |
| Flagellar hook-associated 2-like | CUST_1524_PI425702210 | 3.8 |
| Cell wall/membrane/envelope biogenesis | | |
| NAD-dependent epimerase/dehydratase | CUST_2831_PI425702210 | 3.1 |
| Alginate biosynthesis protein (AlgJ) | CUST_949_PI425702210 | 2.7 |
| Sulfatase | CUST_4086_PI425702210 | 2.4 |
| NLP/P60 | CUST_5295_PI425702210 | 2.3 |

**Table 4** (continued)

| COG class and gene description | Probe name | FC |
|---|---|---|
| NlpC/P60 family protein | CUST_6869_PI425702210 | 2.2 |
| DTDP-4-dehydrorhamnose 3,5-epimerase | CUST_1507_PI425702210 | 2.2 |
| Outer membrane porin | CUST_3601_PI425702210 | 2.1 |
| Defence mechanisms | | |
| Secretion protein (HlyD) | CUST_2648_PI425702210 | 3.6 |
| Intracellular trafficking, secretion and vesicular transport | | |
| ExbB, uptake of enterochelin; TonB-dependent uptake of B colicins (P) | CUST_6908_PI425702210 | 3.9 |
| MotA/TolQ/ExbB proton channel | CUST_5531_PI425702210 | 3.7 |
| Import inner membrane translocase, subunit Tim44 | CUST_5621_PI425702210 | 2.6 |
| Biopolymer transport protein ExbD/TolR | CUST_5532_PI425702210 | 2.1 |
| Post-translational modification, protein turnover, chaperones | | |
| 1-Cys peroxiredoxin | CUST_5394_PI425702210 | 7.3 |
| Alkyl hydroperoxide reductase/thiol specific antioxidant/Mal allergen | CUST_2913_PI425702210 | 4.1 |
| Periplasmic protein thiol:disulfide oxidoreductase (DsbE) | CUST_1578_PI425702210 | 2.9 |
| Thiol-disulfide interchange protein (CcmG) | CUST_6095_PI425702210 | 2.5 |
| Signal transduction mechanisms | | |
| Chemotaxis sensory transducer | CUST_4170_PI425702210 | 7.9 |
| Heavy metal sensor signal transduction histidine kinase | CUST_201_PI425702210 | 2.5 |
| Information storage and processing (18 %) | | |
| Transcription | | |
| Sigma-24 (FecI) | CUST_3919_PI425702210 | 4.8 |
| Transcriptional regulator, IclR family | CUST_906_PI425702210 | 4.4 |
| Sigma-24 (FecI) | CUST_922_PI425702210 | 2.7 |
| SOS-response transcriptional repressor (LexA) | CUST_3850_PI425702210 | 2.4 |
| Transcriptional regulator, DeoR family | CUST_4511_PI425702210 | 2.3 |
| Sigma-32 (RpoH) | CUST_5312_PI425702210 | 2.3 |
| Transcriptional regulator, GntR family | CUST_3125_PI425702210 | 2.2 |
| Transcriptional regulator, AraC family | CUST_3744_PI425702210 | 2.1 |
| Translation, ribosomal structure and biogenesis | | |
| GCN5-related *N*-acetyltransferase | CUST_2838_PI425702210 | 10.9 |
| GCN5-related *N*-acetyltransferase | CUST_2605_PI425702210 | 2.8 |
| SSU ribosomal protein S6P modification protein | CUST_260_PI425702210 | 2.6 |
| FlaG | CUST_6084_PI425702210 | 2.5 |
| GCN5-related *N*-acetyltransferase | CUST_1517_PI425702210 | 2.0 |
| Metabolism (31 %) | | |
| Amino acid transport and metabolism | | |
| Binding-protein-dependent transport systems IMC | CUST_5346_PI425702210 | 2.9 |
| Argininosuccinate synthase | CUST_1895_PI425702210 | 2.6 |
| Extracellular solute-binding protein, family 1 | CUST_5408_PI425702210 | 2.3 |
| Binding-protein-dependent transport systems IMC | CUST_252_PI425702210 | 2.2 |
| Coenzyme transport and metabolism | | |
| Molybdopterin biosynthesis MoeA protein | CUST_2143_PI425702210 | 2.2 |
| Energy production and conversion | | |
| NADH:flavin oxidoreductase/NADH oxidase | CUST_1279_PI425702210 | 18.4 |

**Table 4** (continued)

| COG class and gene description | Probe name | FC |
|---|---|---|
| Luciferase-like protein | CUST_98_PI425702210 | 2.4 |
| Cytochrome c5 | CUST_5822_PI425702210 | 2.3 |
| Succinate semialdehyde dehydrogenase | CUST_185_PI425702210 | 2.2 |
| Malate synthase G | CUST_5161_PI425702210 | 2.1 |
| Inorganic ion transport and metabolism | | |
| NLPA lipoprotein | CUST_221_PI425702210 | 17.2 |
| Thiosulfate-binding protein | CUST_192_PI425702210 | 3.5 |
| Nitrate ABC transporter, periplasmicnitrate-binding protein (P) | CUST_3145_PI425702210 | 3.0 |
| Sulfate transport system permease protein 1 | CUST_195_PI425702210 | 2.9 |
| NLPA lipoprotein | CUST_67_PI425702210 | 2.8 |
| Taurine ABC transporter, periplasmic binding protein | CUST_5396_PI425702210 | 2.7 |
| Taurine ABC transporter, periplasmic binding protein | CUST_254_PI425702210 | 2.6 |
| Integral membrane protein (TerC) | CUST_3078_PI425702210 | 2.3 |
| FecR (P) | CUST_921_PI425702210 | 2.1 |
| Lipid transport and metabolism | | |
| Acyl-phosphate glycerol-3-phosphate acyltransferase | CUST_5123_PI425702210 | 4.0 |
| Phospholipid/glycerol acyltransferase | CUST_1660_PI425702210 | 3.0 |
| Secondary metabolites biosynthesis, transport and catabolism | | |
| Catechol 1,2-dioxygenase | CUST_2318_PI425702210 | 2.3 |
| Poorly characterized (15 %) | | |
| General function prediction only | | |
| Pyridine nucleotide-disulfide oxidoreductase, class-II, active site | CUST_2912_PI425702210 | 8.6 |
| Bile acid:sodium symporter | CUST_3239_PI425702210 | 6.7 |
| Transport-associated protein | CUST_4796_PI425702210 | 4.5 |
| Transthyretin | CUST_198_PI425702210 | 4.4 |
| NADPH-dependent FMN reductase | CUST_2387_PI425702210 | 3.8 |
| ABC transporter-like protein | CUST_4159_PI425702210 | 2.7 |
| FxsA cytoplasmic membrane protein | CUST_4481_PI425702210 | 2.3 |
| LamB/YcsF family protein | CUST_1400_PI425702210 | 2.3 |
| Formate dehydrogenase, subunit FdhD | CUST_278_PI425702210 | 2.1 |
| Ankyrin | CUST_1724_PI425702210 | 2.0 |
| Function unknown | | |
| YceI-like family protein | CUST_5262_PI425702210 | 2.5 |
| Unclassified (8 %) | | |
| Lipopolysaccharide kinase | CUST_4473_PI425702210 | 5.9 |
| Heptose kinase (WapQ) (P) | CUST_6729_PI425702210 | 5.2 |
| $H^+$-transporting two-sector ATPase,delta/epsilon subunit | CUST_5700_PI425702210 | 2.7 |
| HvnB; halovibrin | CUST_2877_PI425702210 | 2.7 |
| YcfA-like protein | CUST_5866_PI425702210 | 2.3 |
| P-loop ATPase protein UPF0042 (P) | CUST_851_PI425702210 | 2.1 |

observed the repression of genes involved in signal transduction: genes encoding chemotaxis transducers and histidine kinases. Unlike MD treatment, 4MD treatment resulted in the repression of sulfatases, sulfate

**Table 5** *P. fluorescens* genes differentially ($p<0.01$) expressed over exposure time under 4MD metal treatment

| COG and gene description | Probe name | FC |
|---|---|---|
| Cluster I (repressed): 356 genes | | |
| Cellular processes and signalling (19 %) | | |
| Cell cycle control, cell division, chromosome partitioning | | |
| Filamentation induced by cAMP protein Fic | CUST_4982_PI425702210 | 3.2 |
| Chromosome segregation DNA-binding protein | CUST_5709_PI425702210 | 2.8 |
| Metallophosphoesterase | CUST_2188_PI425702210 | 2.4 |
| Cell motility | | |
| Pilus assembly protein (CpaE) | CUST_645_PI425702210 | 4.4 |
| Type II secretion system protein E | CUST_644_PI425702210 | 3.2 |
| Type I secretion outer membrane protein (TolC) | CUST_2671_PI425702210 | 2.5 |
| CheW protein | CUST_4632_PI425702210 | 2.3 |
| Flagellar transport protein (FliP) | CUST_1544_PI425702210 | 2.1 |
| NolW-like protein | CUST_3584_PI425702210 | 2.1 |
| Cell wall/membrane/envelope biogenesis | | |
| Outer membrane porin | CUST_1274_PI425702210 | 3.9 |
| Aquaporin | CUST_4509_PI425702210 | 3.7 |
| Sulfatase | CUST_4477_PI425702210 | 3.6 |
| Rod shape-determining protein (RodA) | CUST_4947_PI425702210 | 3.5 |
| Alginate biosynthesis protein (AlgJ) | CUST_949_PI425702210 | 3.3 |
| Alginate biosynthesis protein (Alg8) | CUST_957_PI425702210 | 3.1 |
| General substrate transporter | CUST_2891_PI425702210 | 2.9 |
| Glycosyl transferase, family 39 | CUST_2823_PI425702210 | 2.9 |
| Alginate biosynthesis protein (Alg8) | CUST_5979_PI425702210 | 2.9 |
| OmpA/MotB | CUST_1225_PI425702210 | 2.8 |
| Muconate cycloisomerase | CUST_2316_PI425702210 | 2.8 |
| Glycosyl transferase, family 39 | CUST_2824_PI425702210 | 2.8 |
| Alginate biosynthesis protein (Alg44) | CUST_956_PI425702210 | 2.6 |
| OmpA/MotB | CUST_511_PI425702210 | 2.4 |
| Alginate biosynthesis protein algK precursor | CUST_5978_PI425702210 | 2.4 |
| BCCT transporter | CUST_5219_PI425702210 | 2.3 |
| Lipoprotein (P) | CUST_40_PI425702210 | 2.3 |
| Peptidase M23B | CUST_5077_PI425702210 | 2.3 |
| OmpA/MotB | CUST_4496_PI425702210 | 2.3 |
| Outer membrane porin | CUST_3601_PI425702210 | 2.3 |
| Lipoprotein (P) | CUST_4004_PI425702210 | 2.2 |
| Outer membrane lipoprotein | CUST_6865_PI425702210 | 2.1 |
| UDP-glucose/GDP-mannose dehydrogenase | CUST_958_PI425702210 | 2.1 |
| AsmA family protein | CUST_564_PI425702210 | 2.1 |
| Sulfatase | CUST_2573_PI425702210 | 2.1 |
| Nucleotidyl transferase | CUST_5490_PI425702210 | 2.0 |
| Defence mechanisms | | |
| Drug resistance transporter EmrB/QacA subfamily | CUST_3625_PI425702210 | 5.6 |
| Secretion protein (HlyD) | CUST_2648_PI425702210 | 2.9 |
| Peptidase C39, bacteriocin processing | CUST_2584_PI425702210 | 2.8 |

**Table 5** (continued)

| COG and gene description | Probe name | FC |
|---|---|---|
| Secretion protein HlyD | CUST_2206_PI425702210 | 2.6 |
| Natural resistance-associated macrophage protein | CUST_2065_PI425702210 | 2.4 |
| Secretion protein (HlyD) | CUST_3626_PI425702210 | 2.3 |
| Secretion protein (HlyD) | CUST_2881_PI425702210 | 2.1 |
| Secretion protein (HlyD) | CUST_171_PI425702210 | 2.1 |
| Intracellular trafficking, secretion, and vesicular transport | | |
| MotA/TolQ/ExbB proton channel | CUST_217_PI425702210 | 3.4 |
| Protein translocase subunit (YidC) | CUST_5714_PI425702210 | 2.2 |
| Post-translational modification, protein turnover, chaperones | | |
| Cytochrome oxidase assembly | CUST_72_PI425702210 | 4.7 |
| Heat shock protein (YegD) | CUST_574_PI425702210 | 2.9 |
| HupE/UreJ protein | CUST_558_PI425702210 | 2.8 |
| Signal peptide protein (P) | CUST_6052_PI425702210 | 2.7 |
| Peptidase S1 and S6, chymotrypsin/Hap | CUST_872_PI425702210 | 2.2 |
| Signal transduction mechanisms | | |
| Diguanylate phosphodiesterase (EALdomain) (P) | CUST_3950_PI425702210 | 4.9 |
| Response regulator/ggdef domain protein | CUST_6233_PI425702210 | 4.3 |
| Periplasmic sensor signal transduction histidine kinase | CUST_1350_PI425702210 | 4.2 |
| Protein tyrosine/serine phosphatase | CUST_87_PI425702210 | 4.1 |
| Predicted signal transduction protein | CUST_187_PI425702210 | 4.0 |
| Chemotaxis sensory transducer | CUST_2543_PI425702210 | 3.8 |
| Sensor protein (CpxA) | CUST_6680_PI425702210 | 3.5 |
| Chemotaxis sensory transducer | CUST_1413_PI425702210 | 3.3 |
| Chemotaxis sensory transducer | CUST_3751_PI425702210 | 3.0 |
| Histidine kinase, hamp region:bacterial chemotaxis sensory transducer | CUST_5894_PI425702210 | 2.8 |
| Chemotaxis sensory transducer | CUST_445_PI425702210 | 2.8 |
| Chemotaxis sensory transducer | CUST_3753_PI425702210 | 2.5 |
| Transcriptional activator protein (PfeR) | CUST_6681_PI425702210 | 2.5 |
| Diguanylate cyclase/phosphodiesterase with PAS/PAC sensor(s) | CUST_4854_PI425702210 | 2.5 |
| Diguanylate cyclase with PAS/PAC sensor | CUST_1784_PI425702210 | 2.4 |
| Periplasmic sensor signal transduction histidine kinase | CUST_4198_PI425702210 | 2.3 |
| Chemotaxis sensory transducer | CUST_786_PI425702210 | 2.2 |
| Chemotaxis sensory transducer, Cache sensor | CUST_124_PI425702210 | 2.1 |
| Histidine kinase | CUST_4603_PI425702210 | 2.1 |
| Information storage and processing (16 %) | | |
| Replication, recombination and repair | | |
| Transposase | CUST_6440_PI425702210 | 4.8 |
| Helicase C2 | CUST_1222_PI425702210 | 4.2 |
| DEAD/DEAH box helicase-like | CUST_5226_PI425702210 | 3.6 |
| Holliday junction endonuclease (RuvC) | CUST_4387_PI425702210 | 3.2 |
| ATP-independent RNA helicase (DbpA) | CUST_6858_PI425702210 | 3.0 |
| NUDIX hydrolase | CUST_3578_PI425702210 | 2.8 |
| DNA topoisomerase III | CUST_2985_PI425702210 | 2.6 |
| Exonuclease (RecJ) | CUST_1032_PI425702210 | 2.3 |

**Table 5** (continued)

| COG and gene description | Probe name | FC |
|---|---|---|
| DNA polymerase III, delta subunit | CUST_4937_PI425702210 | 2.1 |
| NAD-dependent DNA ligase | CUST_5248_PI425702210 | 2.0 |
| Transcription | | |
| Regulator of chromosome condensation, RCC1 | CUST_3666_PI425702210 | 6.2 |
| Transcriptional regulator, LysR family | CUST_3785_PI425702210 | 4.1 |
| HTH-type transcriptional regulator (PrtR) | CUST_6014_PI425702210 | 3.9 |
| Transcriptional regulator, AsnC family | CUST_5131_PI425702210 | 3.6 |
| Sigma-24 (FecI) | CUST_3182_PI425702210 | 3.4 |
| Transcriptional regulator, IclR family | CUST_1291_PI425702210 | 3.2 |
| Sigma-24 (FecI) | CUST_3649_PI425702210 | 3.1 |
| Negative transcriptional regulator | CUST_5609_PI425702210 | 3.1 |
| Transcriptional regulator, AraC family with amidase-like domain | CUST_4675_PI425702210 | 3.1 |
| Transcriptional regulator, LysR family | CUST_5492_PI425702210 | 3.0 |
| Transcriptional regulator, LysR family | CUST_1453_PI425702210 | 2.9 |
| Sigma-24 (FecI) | CUST_3420_PI425702210 | 2.9 |
| Periplasmic binding protein/LacI transcriptional regulator | CUST_4119_PI425702210 | 2.7 |
| Transcriptional regulator, LysR family | CUST_1181_PI425702210 | 2.6 |
| Two-component heavy metal response transcriptional regulator, winged helix family | CUST_3363_PI425702210 | 2.6 |
| Sigma-54 specific transcriptional regulator with PAS/Fis DNA-binding domains | CUST_3061_PI425702210 | 2.5 |
| Transcriptional regulator, AraC family | CUST_2301_PI425702210 | 2.5 |
| Sigma-24 (FecI) | CUST_1853_PI425702210 | 2.5 |
| Transcriptional regulator, LysR family | CUST_2182_PI425702210 | 2.5 |
| Sel1-like repeat | CUST_955_PI425702210 | 2.4 |
| ECF-family sigma factor+ | CUST_6142_PI425702210 | 2.4 |
| Two component transcriptional regulator, winged helix family | CUST_2080_PI425702210 | 2.4 |
| Transcriptional regulator, LysR family | CUST_3341_PI425702210 | 2.4 |
| Transcriptional regulator, LacI family | CUST_1917_PI425702210 | 2.4 |
| Transcriptional regulator, AsnC family | CUST_5647_PI425702210 | 2.4 |
| Transcriptional regulator, TetR family | CUST_681_PI425702210 | 2.3 |
| Transcriptional regulator, LysR family | CUST_3352_PI425702210 | 2.3 |
| Transcriptional regulator, TetR family | CUST_3428_PI425702210 | 2.3 |
| Transcriptional regulator, LysR family | CUST_3876_PI425702210 | 2.3 |
| Nucleoside diphosphate pyrophosphatase | CUST_493_PI425702210[a] | 2.3 |
| Transcriptional regulator, AraC family | CUST_4265_PI425702210 | 2.2 |
| Transcriptional regulator, LysR family | CUST_3635_PI425702210 | 2.2 |
| Transcriptional regulator, LysR family | CUST_487_PI425702210 | 2.2 |
| Transcriptional regulator, GntR family | CUST_1005_PI425702210 | 2.2 |
| Transcriptional regulator ArgR | CUST_6697_PI425702210 | 2.1 |
| Transcriptional regulator, DeoR family | CUST_4511_PI425702210 | 2.1 |
| Transcriptional regulator, LysR family | CUST_4569_PI425702210 | 2.1 |
| Transcriptional regulator, GntR family | CUST_4857_PI425702210 | 2.1 |
| Two component transcriptional regulator, LuxR family | CUST_4493_PI425702210 | 2.0 |
| Transcriptional regulator, MerR family | CUST_711_PI425702210 | 2.0 |

**Table 5** (continued)

| COG and gene description | Probe name | FC |
|---|---|---|
| Translation, ribosomal structure and biogenesis | | |
| GCN5-related *N*-acetyltransferase | CUST_2605_PI425702210 | 3.9 |
| 23S rRNA (uracil-5-)-methyltransferase (RumA) | CUST_4196_PI425702210 | 3.1 |
| Acetyltransferase, GNAT family | CUST_6024_PI425702210 | 2.9 |
| Translation initiation factor 2B subunit I family (IF-2BI) | CUST_4057_PI425702210 | 2.6 |
| Pseudouridylate synthase | CUST_1125_PI425702210 | 2.3 |
| Acetyltransferase, GNAT family | CUST_3631_PI425702210 | 2.1 |
| Endoribonuclease L-PSP | CUST_2372_PI425702210 | 2.1 |
| Metabolism (45 %) | | |
| Amino acid transport and metabolism | | |
| Binding-protein-dependent transport systems IMC | CUST_252_PI425702210 | 7.8 |
| Inner-membrane translocator | CUST_587_PI425702210 | 5.7 |
| Shikimate 5-dehydrogenase | CUST_6809_PI425702210 | 4.3 |
| Lysine exporter protein (LYSE/YGGA) | CUST_1319_PI425702210 | 4.2 |
| Binding-protein-dependent transport systems IMC | CUST_5346_PI425702210 | 4.2 |
| Aa ABC transporter, permease protein,3-TM region, His/Glu/Gln/Arg/opine | CUST_5436_PI425702210 | 4.1 |
| Aspartate kinase | CUST_924_PI425702210 | 4.0 |
| Binding-protein-dependent transport systems IMC | CUST_5590_PI425702210 | 3.7 |
| Binding-protein-dependent transport systems IMC | CUST_140_PI425702210 | 3.7 |
| Binding-protein-dependent transport systems IMC | CUST_1679_PI425702210 | 3.7 |
| Lysine exporter protein (LYSE/YGGA) | CUST_5648_PI425702210 | 3.6 |
| Molybdate ABC transporter, permease protein | CUST_2930_PI425702210 | 3.5 |
| Binding-protein-dependent transport systems IMC | CUST_5390_PI425702210 | 3.5 |
| Binding-protein-dependent transport systems IMC | CUST_2826_PI425702210 | 3.4 |
| Aa ABC transporter, permease protein,3-TM region, His/Glu/Gln/Arg/opine | CUST_223_PI425702210 | 3.4 |
| Phosphate ABC transporter permease protein | CUST_6924_PI425702210 | 3.4 |
| Lysine exporter protein (LYSE/YGGA) | CUST_2152_PI425702210 | 3.3 |
| Agmatinase | CUST_1452_PI425702210 | 3.3 |
| Glutamine transport system permease protein (GlnP) | CUST_5850_PI425702210 | 3.2 |
| Aa ABC transporter, permease protein,3-TM region, His/Glu/Gln/Arg/opine | CUST_3074_PI425702210 | 3.2 |
| Allophanate hydrolase subunit 2 | CUST_1335_PI425702210 | 3.2 |
| Serine transporter | CUST_916_PI425702210 | 3.0 |
| Aa ABC transporter, permease protein,3-TM region, His/Glu/Gln/Arg/opine | CUST_224_PI425702210 | 2.9 |
| Agmatinase | CUST_2565_PI425702210 | 2.9 |
| Binding-protein-dependent transport systems IMC | CUST_3058_PI425702210 | 2.9 |
| L-Aspartate aminotransferase | CUST_2893_PI425702210 | 2.8 |
| Binding-protein-dependent transport systems IMC | CUST_1678_PI425702210 | 2.8 |
| Oligopeptide/dipeptide ABC transporter, ATP-binding protein-like | CUST_807_PI425702210 | 2.7 |
| Inner-membrane translocator | CUST_551_PI425702210 | 2.6 |
| ABC transporter permease protein (Y4oQ) (P) | CUST_6332_PI425702210 | 2.6 |
| Binding-protein-dependent transport systems IMC | CUST_2628_PI425702210 | 2.5 |
| Aromatic Aa beta-eliminating lyase/threonine aldolase | CUST_1089_PI425702210 | 2.5 |
| Argininosuccinate synthase | CUST_1895_PI425702210 | 2.5 |

**Table 5** (continued)

| COG and gene description | Probe name | FC |
|---|---|---|
| Urease, beta-subunit | CUST_579_PI425702210 | 2.5 |
| ABC transporter, permease protein, 3-TM region, His/Glu/Gln/Arg/opine | CUST_2841_PI425702210 | 2.4 |
| Binding-protein-dependent transport systems IMC | CUST_5101_PI425702210 | 2.4 |
| Binding-protein-dependent transport systems IMC | CUST_4346_PI425702210 | 2.4 |
| Extracellular solute-binding protein, family 3 | CUST_3076_PI425702210 | 2.3 |
| Glyoxalase/bleomycin resistance protein/dioxygenase | CUST_722_PI425702210 | 2.2 |
| Binding-protein-dependent transport systems IMC | CUST_3059_PI425702210 | 2.1 |
| Transglutaminase-like domain protein | CUST_1732_PI425702210 | 2.1 |
| Inner-membrane translocator | CUST_552_PI425702210 | 2.1 |
| Aminotransferase, class I and II | CUST_2685_PI425702210 | 2.1 |
| Branched-chain Aa transport system II carrier protein | CUST_1614_PI425702210 | 2.0 |
| Carbohydrate transport and metabolism | | |
| Glycerol-3-phosphate transporter | CUST_5427_PI425702210 | 6.7 |
| Shikimate dehydrogenase | CUST_4881_PI425702210 | 4.7 |
| PfkB | CUST_2890_PI425702210 | 4.3 |
| Major facilitator superfamily MFS 1 | CUST_3691_PI425702210 | 4.2 |
| Aldose 1-epimerase | CUST_4343_PI425702210 | 4.0 |
| Gluconolactonase | CUST_3437_PI425702210 | 3.9 |
| Drug resistance transporter Bcr/CflA subfamily | CUST_662_PI425702210 | 3.7 |
| Glycerate 2-kinase | CUST_1590_PI425702210 | 3.4 |
| ABC-2 | CUST_4158_PI425702210 | 3.3 |
| Major facilitator superfamily MFS 1 | CUST_3433_PI425702210 | 3.3 |
| Major facilitator superfamily MFS 1 | CUST_3210_PI425702210 | 3.1 |
| Major facilitator superfamily MFS 1 | CUST_3346_PI425702210 | 3.0 |
| TRAP dicarboxylate transporter subunit DctM | CUST_3440_PI425702210 | 2.9 |
| Aldolase, class II | CUST_5970_PI425702210 | 2.7 |
| General substrate transporter | CUST_1313_PI425702210 | 2.7 |
| General substrate transporter | CUST_3842_PI425702210 | 2.6 |
| Class II aldolase/adducin-like | CUST_898_PI425702210 | 2.5 |
| Major facilitator superfamily MFS 1 | CUST_4236_PI425702210 | 2.5 |
| 2-Keto-3-deoxy-phosphogluconate aldolase | CUST_4339_PI425702210 | 2.5 |
| Quinoprotein glucose dehydrogenase | CUST_4555_PI425702210 | 2.4 |
| General substrate transporter | CUST_1251_PI425702210 | 2.4 |
| Sugar isomerase (SIS) | CUST_1003_PI425702210 | 2.3 |
| Major facilitator superfamily transporter | CUST_6703_PI425702210 | 2.2 |
| Major facilitator superfamily MFS_1 | CUST_4885_PI425702210 | 2.2 |
| Ribose transport ATP-binding protein (RbsA) | CUST_6155_PI425702210 | 2.1 |
| Fructose-bisphosphate aldolase | CUST_5240_PI425702210 | 2.1 |
| General substrate transporter | CUST_668_PI425702210 | 2.1 |
| Chromatin structure and dynamics | | |
| Histone deacetylase superfamily | CUST_5606_PI425702210 | 2.6 |
| Coenzyme transport and metabolism | | |
| Sulfur transfer protein, thiamine S (ThiS) | CUST_2138_PI425702210 | 3.1 |
| Nicotinate-nucleotide-dimethylbenzimidazolephosphoribosyltransferase | CUST_1643_PI425702210 | 2.8 |

**Table 5** (continued)

| COG and gene description | Probe name | FC |
|---|---|---|
| Anthranilate synthase, component II | CUST_5093_PI425702210 | 2.5 |
| Probable HesA/MoeB/ThiF family protein | CUST_6004_PI425702210 | 2.3 |
| Adenosylmethionine-8-amino-7-oxononanoateaminotransferase | CUST_5265_PI425702210 | 2.3 |
| Adenosylcobinamide-phosphate synthase | CUST_1639_PI425702210 | 2.2 |
| Energy production and conversion | | |
| FAD dependent oxidoreductase | CUST_3662_PI425702210 | 6.1 |
| Luciferase-like | CUST_5391_PI425702210 | 5.7 |
| Cytochrome c, class I | CUST_5446_PI425702210 | 4.3 |
| L-Lactate permease | CUST_750_PI425702210 | 4.0 |
| Oxidoreductase FAD/NAD(P)-binding | CUST_4866_PI425702210 | 2.8 |
| Oxidoreductase alpha (molybdopterin) subunit | CUST_277_PI425702210 | 2.8 |
| Fumarase | CUST_846_PI425702210 | 2.8 |
| Fumarate hydratase class II 1 | CUST_5960_PI425702210 | 2.6 |
| NADH:flavin oxidoreductase/NADH oxidase | CUST_2468_PI425702210 | 2.4 |
| FAD-dependent oxidoreductase | CUST_2818_PI425702210 | 2.4 |
| Cytochrome c, class I | CUST_1733_PI425702210 | 2.2 |
| Cytochrome c oxidase, subunit III | CUST_76_PI425702210 | 2.1 |
| Cytochrome c, class I | CUST_6119_PI425702210 | 2.1 |
| Inorganic ion transport and metabolism | | |
| ABC transporter, substrate-binding protein, aliphatic sulfonates | CUST_5392_PI425702210 | 6.1 |
| Assimilatory nitrite reductase (NAD(P)H) small subunit | CUST_1775_PI425702210 | 5.4 |
| Sodium/hydrogen exchanger | CUST_444_PI425702210 | 4.3 |
| Sulfate transporter | CUST_81_PI425702210 | 4.3 |
| Potassium-translocating P-type ATPase, B-subunit | CUST_4010_PI425702210 | 3.9 |
| Heme oxygenase | CUST_4358_PI425702210 | 3.8 |
| Phosphate transport system permease protein 2 | CUST_5589_PI425702210 | 3.4 |
| TonB-dependent siderophore receptor | CUST_795_PI425702210 | 3.4 |
| Na/Pi cotransporter II-like | CUST_34_PI425702210 | 3.3 |
| Heavy metal-(Cd/Co/Hg/Pb/Zn)-translocating P-type ATPase | CUST_5349_PI425702210 | 3.1 |
| Periplasmic solute binding protein | CUST_5636_PI425702210 | 3.1 |
| Di-haem cytochrome c peroxidase | CUST_4531_PI425702210 | 3.0 |
| TonB-dependent siderophore receptor | CUST_871_PI425702210 | 3.0 |
| Sulfate ABC transporter, permease protein CysT | CUST_193_PI425702210 | 2.9 |
| $K^+$-dependent Na+/Ca+exchanger protein | CUST_830_PI425702210 | 2.9 |
| FecR (P) | CUST_3767_PI425702210 | 2.8 |
| Magnesium-translocating P-type ATPase | CUST_1855_PI425702210 | 2.8 |
| Possible uncharacterized iron-regulated membrane protein | CUST_6853_PI425702210 | 2.6 |
| FecR (P) | CUST_3779_PI425702210 | 2.6 |
| Arsenical pump membrane protein | CUST_2389_PI425702210 | 2.6 |
| TonB-dependent receptor | CUST_3108_PI425702210 | 2.5 |
| FecR (P) | CUST_5166_PI425702210 | 2.5 |
| Thiosulfate-binding protein | CUST_192_PI425702210 | 2.4 |
| Sodium/hydrogen exchanger | CUST_5255_PI425702210 | 2.4 |
| TonB-dependent siderophore receptor | CUST_920_PI425702210 | 2.4 |

**Table 5** (continued)

| COG and gene description | Probe name | FC |
|---|---|---|
| TonB-dependent siderophore receptor | CUST_1843_PI425702210 | 2.3 |
| Periplasmic protein (P) | CUST_5868_PI425702210 | 2.3 |
| Potassium efflux system protein (PhaE) (P) | CUST_3297_PI425702210 | 2.1 |
| Transport system permease protein | CUST_4777_PI425702210 | 2.1 |
| Intracellular trafficking, secretion, and vesicular transport | | |
| Twin-arginine translocation pathway signal | CUST_1849_PI425702210 | 5.1 |
| Twin-arginine translocation pathway signal | CUST_5157_PI425702210 | 4.1 |
| Lipid transport and metabolism | | |
| Phosphatidylglycerophosphatase | CUST_4991_PI425702210 | 4.0 |
| Acyl-CoA dehydrogenase-like | CUST_449_PI425702210 | 3.9 |
| Coenzyme A transferase | CUST_1265_PI425702210 | 3.6 |
| Acyl-CoA dehydrogenase-like | CUST_3895_PI425702210 | 3.6 |
| Phospholipid/glycerol acyltransferase | CUST_1660_PI425702210 | 3.5 |
| Fatty acid desaturase | CUST_4325_PI425702210 | 3.2 |
| Acyl-CoA dehydrogenase-like | CUST_238_PI425702210 | 3.0 |
| Malonate decarboxylase delta-subunit | CUST_5278_PI425702210 | 2.8 |
| Glycerol-3-phosphate acyltransferase | CUST_1081_PI425702210 | 2.8 |
| Short-chain dehydrogenase/reductase SDR | CUST_1235_PI425702210 | 2.7 |
| 2-Hydroxy-3-oxopropionate reductase | CUST_1591_PI425702210 | 2.6 |
| Glucose 1-dehydrogenase | CUST_1835_PI425702210 | 2.4 |
| Acyl-CoA dehydrogenase-like | CUST_3926_PI425702210 | 2.4 |
| Acetyl-CoA C-acyltransferase | CUST_4074_PI425702210 | 2.4 |
| Short-chain dehydrogenase/reductase SDR | CUST_943_PI425702210 | 2.3 |
| Acyl-CoA dehydrogenase-like | CUST_3634_PI425702210 | 2.3 |
| Lipase, class 3 | CUST_2675_PI425702210 | 2.2 |
| Beta-ketoacyl-acyl carrier protein synthase II | CUST_5887_PI425702210 | 2.2 |
| (Acyl-carrier protein) phosphodiesterase | CUST_2595_PI425702210 | 2.0 |
| Phospholipid/glycerol acyltransferase | CUST_4697_PI425702210 | 2.0 |
| Short-chain dehydrogenase/reductase SDR | CUST_1748_PI425702210 | 2.0 |
| Nucleotide transport and metabolism | | |
| Xanthine/uracil permease | CUST_1696_PI425702210 | 5.1 |
| Permease for cytosine/purines, uracil, thiamine, allantoin | CUST_491_PI425702210 | 3.4 |
| Permease for cytosine/purines, uracil, thiamine, allantoin | CUST_1450_PI425702210 | 3.1 |
| Adenosine deaminase | CUST_669_PI425702210 | 2.3 |
| Methylthioadenosine phosphorylase | CUST_3847_PI425702210 | 2.2 |
| Xanthine/uracil permease | CUST_2562_PI425702210 | 2.1 |
| Permease for cytosine/purines, uracil, thiamine, allantoin | CUST_3254_PI425702210 | 2.0 |
| Secondary metabolites biosynthesis, transport and catabolism | | |
| Aa adenylation domain protein | CUST_1842_PI425702210 | 4.4 |
| Aa adenylation domain protein | CUST_2203_PI425702210 | 4.3 |
| Benzoate transport | CUST_1263_PI425702210 | 4.0 |
| Multicopper oxidase, type 2 | CUST_1996_PI425702210 | 3.7 |
| 4′-Phosphopantetheinyl transferase | CUST_4221_PI425702210 | 3.6 |
| Taurine catabolism dioxygenase TauD/TfdA | CUST_137_PI425702210 | 3.5 |

**Table 5** (continued)

| COG and gene description | Probe name | FC |
|---|---|---|
| Gamma-butyrobetaine hydroxylase | CUST_5212_PI425702210 | 2.5 |
| Aa adenylation domain protein | CUST_2204_PI425702210 | 2.5 |
| Fumarylacetoacetate (FAA) hydrolase | CUST_2226_PI425702210 | 2.3 |
| Poorly characterized (17 %) | | |
| Function unknown | | |
| DoxX | CUST_1237_PI425702210 | 5.5 |
| Membrane protein, putative | CUST_428_PI425702210 | 4.2 |
| Membrane protein (P) | CUST_6923_PI425702210 | 3.5 |
| Rhs element Vgr protein | CUST_3399_PI425702210 | 3.4 |
| Fusaric acid resistance protein conserved region | CUST_936_PI425702210 | 3.2 |
| SdiA-regulated | CUST_5362_PI425702210 | 3.0 |
| Membrane protein (P) | CUST_146_PI425702210 | 2.8 |
| Membrane protein (P) | CUST_6744_PI425702210 | 2.7 |
| Allergen V5/Tpx-1-like | CUST_2098_PI425702210 | 2.7 |
| Membrane protein (P) | CUST_6388_PI425702210 | 2.6 |
| Membrane protein (P) | CUST_6740_PI425702210 | 2.6 |
| Outer membrane autotransporter barrel protein | CUST_5612_PI425702210 | 2.5 |
| Adenylate cyclase | CUST_5399_PI425702210 | 2.4 |
| Aromatic-ring-hydroxylating dioxygenase, beta-subunit | CUST_2955_PI425702210 | 2.4 |
| Membrane protein (P) | CUST_435_PI425702210 | 2.4 |
| Paraquat-inducible protein A | CUST_2430_PI425702210 | 2.3 |
| YaiI/YqxD family protein | CUST_6892_PI425702210 | 2.3 |
| DoxX | CUST_3997_PI425702210 | 2.3 |
| Cobalt transporter subunit (CbtA) (P) | CUST_3085_PI425702210 | 2.1 |
| General function prediction only | | |
| ABC transporter-like | CUST_5389_PI425702210 | 7.4 |
| ABC transporter-like | CUST_253_PI425702210 | 5.3 |
| ABC transporter-like | CUST_38_PI425702210 | 4.7 |
| Sodium:dicarboxylate symporter | CUST_2233_PI425702210 | 4.5 |
| ABC transporter-like | CUST_5347_PI425702210 | 4.0 |
| ATP-binding subunit (P) | CUST_5925_PI425702210 | 3.6 |
| Cytochrome c oxidase assembly protein CtaG/Cox11 | CUST_77_PI425702210 | 3.5 |
| ABC transporter-like | CUST_240_PI425702210 | 3.4 |
| ABC transporter-like | CUST_585_PI425702210 | 3.2 |
| Xanthine/uracil/vitamin C permease | CUST_1706_PI425702210 | 2.9 |
| ATP:cob(I)alamin adenosyltransferase | CUST_4402_PI425702210 | 2.8 |
| Metal dependent phosphohydrolase | CUST_5378_PI425702210 | 2.8 |
| GCN5-related *N*-acetyltransferase | CUST_1187_PI425702210 | 2.7 |
| RarD protein | CUST_5159_PI425702210 | 2.7 |
| ABC transporter-like | CUST_3060_PI425702210 | 2.7 |
| Von Willebrand factor, type A | CUST_181_PI425702210 | 2.6 |
| Radical SAM | CUST_3453_PI425702210 | 2.6 |
| FAD-dependent pyridine nucleotide-disulfideoxidoreductase | CUST_5577_PI425702210 | 2.6 |
| GCN5-related *N*-acetyltransferase | CUST_5610_PI425702210 | 2.6 |

**Table 5** (continued)

| COG and gene description | Probe name | FC |
|---|---|---|
| Alpha/beta hydrolase fold family | CUST_5152_PI425702210 | 2.5 |
| HD domain protein | CUST_6879_PI425702210 | 2.5 |
| TRNA modification GTPase (TrmE) | CUST_5713_PI425702210 | 2.5 |
| Pirin-like protein | CUST_2660_PI425702210 | 2.4 |
| ABC transporter-like | CUST_5004_PI425702210 | 2.4 |
| ABC transporter-like | CUST_3617_PI425702210 | 2.4 |
| ABC transporter-like | CUST_1915_PI425702210 | 2.4 |
| Binding-protein-dependent transport systems IMC | CUST_5379_PI425702210 | 2.4 |
| ABC transporter-like | CUST_2842_PI425702210 | 2.3 |
| Creatininase | CUST_6957_PI425702210 | 2.3 |
| ABC transporter-like | CUST_974_PI425702210 | 2.3 |
| Cytochrome c assembly protein | CUST_5990_PI425702210 | 2.3 |
| Pyocin R2_PP, lytic enzyme | CUST_1169_PI425702210 | 2.2 |
| Aminoglycoside phosphotransferase | CUST_5158_PI425702210 | 2.2 |
| Von Willebrand factor type A domain protein | CUST_5847_PI425702210 | 2.2 |
| ABC transporter-like | CUST_4776_PI425702210 | 2.2 |
| ABC transporter-like | CUST_2422_PI425702210 | 2.1 |
| Zinc-containing alcohol dehydrogenase superfamily | CUST_4098_PI425702210 | 2.1 |
| GCN5-related *N*-acetyltransferase | CUST_3429_PI425702210 | 2.1 |
| 2-Octaprenylphenol hydroxylase | CUST_383_PI425702210 | 2.1 |
| Carbonic anhydrase | CUST_6357_PI425702210 | 2.1 |
| Phenylacetic acid degradation-like protein | CUST_3520_PI425702210 | 2.0 |
| Unclassified (3 %) | | |
| Abortive infection protein | CUST_1829_PI425702210 | 5.0 |
| tRNA-Met-CAT | CUST_5790_PI425702210 | 3.3 |
| RhaT protein | CUST_6653_PI425702210 | 3.0 |
| Carbohydrate binding and sugar hydrolysis protein | CUST_953_PI425702210 | 2.6 |
| Malonate/sodium symporter MadM subunit | CUST_5272_PI425702210 | 2.3 |
| Poly(beta-D-mannuronate) C5 epimerase precursor | CUST_5977_PI425702210 | 2.2 |
| (1,4)-Alpha-D-glucan 1-alpha-D-glucosylmutase | CUST_2535_PI425702210 | 2.2 |
| Selenocysteine lyase (P) | CUST_6005_PI425702210 | 2.1 |
| Dethiobiotin synthase | CUST_5150_PI425702210 | 2.1 |
| Poly(beta-D-mannuronate) lyase | CUST_951_PI425702210 | 2.1 |
| Cyanide insensitive terminal oxidase | CUST_6508_PI425702210 | 2.0 |
| Cluster II (overexpressed): 375 genes | | |
| Cellular processes and signalling (26 %) | | |
| Cell cycle control, cell division, chromosome partitioning | | |
| Cell division protein FtsZ | CUST_4646_PI425702210 | 2.6 |
| Cell motility | | |
| Type IV pilus assembly protein (PilW) | CUST_4823_PI425702210 | 2.8 |
| Chemotaxis phosphatase (CheZ) | CUST_1560_PI425702210 | 2.7 |
| General secretion pathway protein H | CUST_4797_PI425702210 | 2.7 |
| Flagellin-like protein | CUST_1522_PI425702210 | 2.6 |

**Table 5** (continued)

| COG and gene description | Probe name | FC |
|---|---|---|
| Possible CheA Signal transduction histidine kinases (STHK) | CUST_5456_PI425702210 | 2.5 |
| Methyl-accepting chemotaxis protein | CUST_6317_PI425702210 | 2.4 |
| Methyl-accepting chemotaxis protein (MCP) signalling domain | CUST_5872_PI425702210 | 2.1 |
| CheA signal transduction histidine kinases (STHK) | CUST_1561_PI425702210 | 2.0 |
| Cell wall/membrane/envelope biogenesis | | |
| TonB-like protein | CUST_3720_PI425702210 | 3.2 |
| Macrolide-specific efflux protein MacA precursor | CUST_6140_PI425702210 | 3.1 |
| Rhs family protein | CUST_1746_PI425702210 | 3.0 |
| Peptidase S45, penicillin amidase | CUST_2552_PI425702210 | 2.9 |
| Insecticidal toxin protein (P) | CUST_4434_PI425702210 | 2.9 |
| Nitrilase/cyanide hydratase and apolipoprotein *N*-acyltransferase | CUST_4573_PI425702210 | 2.8 |
| AsmA | CUST_319_PI425702210 | 2.7 |
| Mannose-1-phosphate guanylyltransferase (GDP) | CUST_5658_PI425702210 | 2.6 |
| RND efflux system, outer membrane lipoprotein (NodT) | CUST_2745_PI425702210 | 2.6 |
| Peptidoglycan-binding (LysM) | CUST_280_PI425702210 | 2.5 |
| Insecticidal toxin complex protein (TccC1) | CUST_6727_PI425702210 | 2.4 |
| DTDP-glucose 4,6-dehydratase | CUST_4036_PI425702210 | 2.4 |
| Outer membrane autotransporter | CUST_6402_PI425702210 | 2.3 |
| UDP-glucose 6-dehydrogenase (P) | CUST_6175_PI425702210 | 2.3 |
| UDP-*N*-acetylmuramoylalanine-D-glutamate ligase | CUST_4653_PI425702210 | 2.3 |
| OmpA/MotB | CUST_4379_PI425702210 | 2.3 |
| UDP-glucose/GDP-mannose dehydrogenase | CUST_2019_PI425702210 | 2.3 |
| UDP-3-*O*-[3-hydroxymyristoyl]*N*-acetylglucosamine deacetylase | CUST_4645_PI425702210 | 2.3 |
| Sugar transferase | CUST_3808_PI425702210 | 2.2 |
| MscS mechanosensitive ion channel | CUST_1683_PI425702210 | 2.2 |
| Organic solvent tolerance protein | CUST_5110_PI425702210 | 2.1 |
| NAD-dependent epimerase/dehydratase | CUST_718_PI425702210 | 2.1 |
| OmpA family protein | CUST_5937_PI425702210 | 2.0 |
| OmpF | CUST_1772_PI425702210 | 2.0 |
| Peptidase M10A and M12B, matrixin andadamalysin | CUST_4683_PI425702210 | 2.0 |
| Defence mechanisms | | |
| Beta-lactamase-like | CUST_4452_PI425702210 | 3.0 |
| Type I secretion membrane fusion protein (HlyD) | CUST_1455_PI425702210 | 2.9 |
| Beta-lactamase | CUST_3706_PI425702210 | 2.7 |
| Beta-lactamase-like | CUST_2271_PI425702210 | 2.6 |
| Secretion protein (HlyD) | CUST_1277_PI425702210 | 2.4 |
| Secretion protein (HlyD) | CUST_2130_PI425702210 | 2.3 |
| Secretion protein HlyD | CUST_228_PI425702210 | 2.2 |
| Secretion protein HlyD | CUST_1076_PI425702210 | 2.0 |
| Intracellular trafficking, secretion and vesicular transport | | |
| General secretion pathway protein I | CUST_3164_PI425702210 | 2.6 |
| MotA/TolQ/ExbB proton channel | CUST_2487_PI425702210 | 2.1 |
| Post-translational modification, protein turnover, chaperones | | |
| Fkbp-type peptidyl-prolyl *cis-trans* isomerase | CUST_6523_PI425702210 | 3.2 |

**Table 5** (continued)

| COG and gene description | Probe name | FC |
|---|---|---|
| ADP-ribosylation/Crystallin J1 | CUST_3871_PI425702210 | 3.2 |
| Chaperonin (Cpn10) | CUST_4480_PI425702210 | 2.9 |
| Probable ClpA/B-type protease | CUST_3396_PI425702210 | 2.8 |
| GrpE protein | CUST_759_PI425702210 | 2.6 |
| ATP-dependent Clp protease adaptor protein (ClpS) | CUST_3574_PI425702210 | 2.5 |
| ADP-ribosylation/Crystallin J1 | CUST_4115_PI425702210 | 2.5 |
| Curved DNA-binding protein | CUST_5922_PI425702210 | 2.5 |
| Heat shock protein (Hsp70) | CUST_760_PI425702210 | 2.5 |
| ATP-dependent Clp protease adaptor protein (ClpS) | CUST_6546_PI425702210 | 2.5 |
| Alkyl hydroperoxide reductase/Thiol specific antioxidant/Mal allergen | CUST_2913_PI425702210 | 2.4 |
| Chaperonin Cpn60/TCP-1 | CUST_4479_PI425702210 | 2.4 |
| PpiC-type peptidyl-prolyl *cis-trans* isomerase | CUST_5111_PI425702210 | 2.4 |
| Chaperone protein (HtpG) | CUST_6101_PI425702210 | 2.3 |
| ATP-dependent Clp protease proteolytic subunit (ClpP) | CUST_3680_PI425702210 | 2.2 |
| Peptidylprolyl isomerase, FKBP-type | CUST_1666_PI425702210 | 2.2 |
| ATP-dependent Clp protease ATP-binding subunit (ClpA) | CUST_3573_PI425702210 | 2.2 |
| Cytochrome c oxidase cbb3-type, subunit I | CUST_1818_PI425702210 | 2.2 |
| Heat shock protein (Hsp90) | CUST_1620_PI425702210 | 2.1 |
| Fe-S protein assembly chaperone (HscA) | CUST_4586_PI425702210 | 2.1 |
| PIM1 peptidase. Serine peptidase. MEROPS family S16 | CUST_4557_PI425702210 | 2.1 |
| Protein-methionine-*S*-oxide reductase | CUST_2767_PI425702210 | 2.1 |
| Thiol peroxidase (atypical 2-Cys peroxiredoxin) | CUST_2509_PI425702210 | 2.1 |
| Chaperone SurA precursor | CUST_6845_PI425702210 | 2.1 |
| Glutathione-S-transferase-like | CUST_5665_PI425702210 | 2.1 |
| ATP-dependent Clp protease ATP-binding subunit (ClpX) | CUST_3679_PI425702210 | 2.1 |
| Peptidyl-prolyl *cis-trans* isomerase,cyclophilin type | CUST_3623_PI425702210 | 2.0 |
| Replication, recombination and repair | | |
| Resolvase-like | CUST_3484_PI425702210 | 2.6 |
| ATP-dependent helicase (HrpB) | CUST_4913_PI425702210 | 2.6 |
| Transposase for insertion sequence element A | CUST_6586_PI425702210 | 2.5 |
| Integration host factor, alpha-subunit | CUST_1932_PI425702210 | 2.1 |
| Integration host factor, alpha-subunit (IhfA) | CUST_6157_PI425702210 | 2.0 |
| Signal transduction mechanisms | | |
| Chemotaxis sensory transducer, Cache sensor | CUST_352_PI425702210 | 3.1 |
| Diguanylate cyclase (GGDEF domain) (P) | CUST_3782_PI425702210 | 3.0 |
| Ggdef domain protein | CUST_6579_PI425702210 | 3.0 |
| Periplasmic sensor signal transduction histidine kinase | CUST_2079_PI425702210 | 2.8 |
| Phosphoenolpyruvate–protein phosphotransferase | CUST_1002_PI425702210 | 2.8 |
| Cyclic nucleotide-regulated small mechanosensitive ion channel | CUST_1963_PI425702210 | 2.8 |
| Periplasmic sensor hybrid histidine kinase | CUST_3237_PI425702210 | 2.6 |
| PAS/PAC sensor signal transduction histidine kinase | CUST_5105_PI425702210 | 2.6 |
| Anti-sigma-factor antagonist (STAS) | CUST_3118_PI425702210 | 2.6 |
| Anti-sigma-E protein (RseA) | CUST_1358_PI425702210 | 2.5 |
| Chemotaxis sensory transducer | CUST_2590_PI425702210 | 2.4 |

**Table 5** (continued)

| COG and gene description | Probe name | FC |
|---|---|---|
| Chemotaxis sensory transducer | CUST_620_PI425702210 | 2.4 |
| Ggdef | CUST_6652_PI425702210 | 2.3 |
| Diguanylate cyclase/phosphodiesterase withPAS/PAC sensor(s) | CUST_4064_PI425702210 | 2.2 |
| Two-component response regulator (CbrB) | CUST_4786_PI425702210 | 2.2 |
| Carbon storage regulator (CsrA) | CUST_4251_PI425702210 | 2.2 |
| Two-component transcriptional regulator, winged helix family | CUST_5_PI425702210 | 2.1 |
| GTP-binding protein TypA | CUST_345_PI425702210 | 2.1 |
| Two-component response regulator (PilR) | CUST_4818_PI425702210 | 2.1 |
| Response regulator receiver domain protein (CheY) | CUST_3512_PI425702210 | 2.1 |
| Response regulator receiver domain protein (CheY) | CUST_3806_PI425702210 | 2.1 |
| Serine protein kinase (PrkA) (P) | CUST_5117_PI425702210 | 2.0 |
| Periplasmic sensor signal transduction histidine kinase | CUST_3912_PI425702210 | 2.0 |
| Information storage and processing (22 %) | | |
| Replication, recombination and repair | | |
| DNA gyrase subunit A | CUST_4056_PI425702210 | 2.3 |
| Deoxyribodipyrimidine photo-lyase type I | CUST_4714_PI425702210 | 2.0 |
| Single-strand binding protein | CUST_5027_PI425702210 | 2.0 |
| Transcription | | |
| Transcriptional regulator, LysR family | CUST_3507_PI425702210 | 3.4 |
| Transcriptional regulator, LysR family | CUST_2145_PI425702210 | 3.2 |
| Transcriptional regulator, AraC family | CUST_2250_PI425702210 | 3.1 |
| Transcriptional regulator, LysR family | CUST_4301_PI425702210 | 3.0 |
| Transcriptional regulator, LysR family | CUST_2494_PI425702210 | 2.7 |
| NAD-dependent protein deacetylases family | CUST_6186_PI425702210 | 2.6 |
| Transcriptional regulator, LysR family | CUST_2907_PI425702210 | 2.6 |
| Silent information regulator protein (Sir2) | CUST_2072_PI425702210 | 2.6 |
| Sigma-28 (flagella/sporulation) | CUST_1558_PI425702210 | 2.6 |
| Transcriptional regulator (P) | CUST_6819_PI425702210 | 2.6 |
| Transcriptional regulator, LysR family | CUST_4450_PI425702210 | 2.5 |
| Two component transcriptional regulator, LuxR family | CUST_5683_PI425702210 | 2.5 |
| DNA-directed RNA polymerase, beta-subunit | CUST_5064_PI425702210 | 2.5 |
| DNA-directed RNA polymerase, beta-subunit (RpoB) | CUST_6835_PI425702210 | 2.4 |
| Transcriptional regulator, LysR family | CUST_1310_PI425702210 | 2.4 |
| Transcription termination factor Rho | CUST_5432_PI425702210 | 2.4 |
| Transcriptional regulator | CUST_4975_PI425702210 | 2.3 |
| DNA-directed RNA polymerase, alpha-subunit | CUST_5033_PI425702210 | 2.3 |
| Transcriptional regulator, AraC family | CUST_2957_PI425702210 | 2.3 |
| Sigma-24 (FecI) | CUST_3110_PI425702210 | 2.3 |
| Transcriptional regulator, LysR family | CUST_3750_PI425702210 | 2.3 |
| Ribonuclease inhibitor barstar | CUST_2033_PI425702210[a] | 2.2 |
| Transcriptional regulator, TetR family | CUST_2759_PI425702210 | 2.2 |
| Two component transcriptional regulator, LuxR family | CUST_638_PI425702210 | 2.2 |
| Sigma-70 (RpoD) | CUST_5127_PI425702210 | 2.1 |
| Transcriptional regulator, GntR family | CUST_5507_PI425702210 | 2.1 |

**Table 5** (continued)

| COG and gene description | Probe name | FC |
|---|---|---|
| Anti-sigma-28 factor (FlgM) | CUST_4233_PI425702210 | 2.1 |
| Transcriptional regulator, LysR family | CUST_2839_PI425702210 | 2.1 |
| Transcriptional regulator, MarR family | CUST_1464_PI425702210 | 2.1 |
| Transcriptional regulator, TetR family | CUST_2469_PI425702210 | 2.1 |
| Transcriptional regulator, MarR family | CUST_1707_PI425702210 | 2.0 |
| Transcriptional regulator, winged helix family | CUST_1953_PI425702210 | 2.0 |
| Transcriptional regulator | CUST_6454_PI425702210 | 2.0 |
| Transcriptional regulator, LacI family | CUST_789_PI425702210 | 2.0 |
| Translation, ribosomal structure and biogenesis | | |
| Acetyltransferase, GNAT family | CUST_3052_PI425702210 | 3.0 |
| LSU ribosomal protein L13P | CUST_4672_PI425702210 | 2.6 |
| LSU ribosomal protein L21P | CUST_4838_PI425702210 | 2.6 |
| Ribosome modulation factor | CUST_1782_PI425702210 | 2.5 |
| SSU ribosomal protein S10P | CUST_5058_PI425702210 | 2.5 |
| Threonyl-tRNA synthetase/Ser-tRNA(Thr)hydrolase | CUST_1926_PI425702210 | 2.5 |
| LSU ribosomal protein L14P | CUST_5047_PI425702210 | 2.5 |
| SSU ribosomal protein S12P | CUST_5062_PI425702210 | 2.5 |
| LSU ribosomal protein L22P | CUST_5052_PI425702210 | 2.4 |
| SSU ribosomal protein S6P | CUST_532_PI425702210 | 2.4 |
| Glycyl-tRNA synthetase, beta-subunit | CUST_9_PI425702210 | 2.4 |
| Bacterial translation initiation factor 3 (bIF-3) | CUST_1927_PI425702210 | 2.4 |
| LSU ribosomal protein L29P | CUST_5049_PI425702210 | 2.4 |
| SSU ribosomal protein S2P | CUST_1098_PI425702210 | 2.4 |
| Ribosomal protein S7 (RpsG) | CUST_6834_PI425702210 | 2.4 |
| Ribonuclease D | CUST_1383_PI425702210 | 2.4 |
| LSU ribosomal protein L2P | CUST_5054_PI425702210 | 2.4 |
| Methionine aminopeptidase, type I | CUST_3229_PI425702210 | 2.4 |
| Ribosomal protein L36 (RpmJ) | CUST_6829_PI425702210 | 2.3 |
| SSU ribosomal protein S3P | CUST_5051_PI425702210 | 2.3 |
| LSU ribosomal protein L6P | CUST_5042_PI425702210 | 2.3 |
| SSU ribosomal protein S5P | CUST_5040_PI425702210 | 2.3 |
| LSU ribosomal protein L4P | CUST_5056_PI425702210 | 2.2 |
| Ribosomal protein L22 (RplV) | CUST_6832_PI425702210 | 2.2 |
| LSU ribosomal protein L5P | CUST_5045_PI425702210 | 2.2 |
| LSU ribosomal protein L24P | CUST_5046_PI425702210 | 2.2 |
| SSU ribosomal protein S19P | CUST_5053_PI425702210 | 2.2 |
| LSU ribosomal protein L16P | CUST_5050_PI425702210 | 2.2 |
| LSU ribosomal protein L25P | CUST_4732_PI425702210 | 2.2 |
| LSU ribosomal protein L35P | CUST_1928_PI425702210 | 2.2 |
| LSU ribosomal protein L23P | CUST_5055_PI425702210 | 2.2 |
| Endoribonuclease L-PSP | CUST_3251_PI425702210 | 2.2 |
| LSU ribosomal protein L11P | CUST_5068_PI425702210 | 2.2 |
| SSU ribosomal protein S17P | CUST_5048_PI425702210 | 2.2 |
| LSU ribosomal protein L30P | CUST_5039_PI425702210 | 2.2 |

**Table 5** (continued)

| COG and gene description | Probe name | FC |
|---|---|---|
| Methionine aminopeptidase, type I | CUST_1097_PI425702210 | 2.2 |
| Endoribonuclease L-PSP | CUST_2850_PI425702210 | 2.1 |
| SSU ribosomal protein S21P | CUST_5125_PI425702210 | 2.1 |
| LSU ribosomal protein L3P | CUST_5057_PI425702210 | 2.1 |
| SSU ribosomal protein S11P | CUST_5035_PI425702210 | 2.1 |
| Methyltransferase small | CUST_2090_PI425702210 | 2.1 |
| LSU ribosomal protein L15P | CUST_5038_PI425702210 | 2.0 |
| SSU ribosomal protein S4P | CUST_5034_PI425702210 | 2.0 |
| SSU ribosomal protein S20P | CUST_4833_PI425702210 | 2.0 |
| Aspartyl/glutamyl-tRNA(Asn/Gln)amidotransferase subunit A | CUST_833_PI425702210 | 2.0 |
| Metabolism (37 %) | | |
| Amino acid transport and metabolism | | |
| Extracellular solute-binding protein, family 1 | CUST_3613_PI425702210 | 3.1 |
| Phosphoadenosine phosphosulfate reductase | CUST_736_PI425702210 | 3.1 |
| Glycine dehydrogenase (decarboxylating) alpha-subunit/beta-subunit | CUST_4370_PI425702210 | 3.0 |
| Arginine deiminase | CUST_4364_PI425702210 | 3.0 |
| Glyoxalase I | CUST_2911_PI425702210 | 2.9 |
| Ornithine carbamoyltransferase | CUST_4365_PI425702210 | 2.9 |
| Arginine/ornithine antiporter | CUST_4363_PI425702210 | 2.9 |
| Extracellular solute-binding protein, family 5 | CUST_2173_PI425702210 | 2.7 |
| Pyridoxal-5′-phosphate-dependent enzyme, beta-subunit | CUST_2217_PI425702210 | 2.6 |
| 4-Hydroxyphenylpyruvate dioxygenase | CUST_2905_PI425702210 | 2.5 |
| Arginine/ornithine antiporter | CUST_6714_PI425702210 | 2.5 |
| Serine hydroxymethyltransferase | CUST_4855_PI425702210 | 2.5 |
| Glutathionylspermidine synthase | CUST_5026_PI425702210 | 2.4 |
| Spermidine/putrescine ABC transporter ATP-binding subunit | CUST_3126_PI425702210 | 2.3 |
| Carbamate kinase | CUST_4366_PI425702210 | 2.3 |
| Peptidase S58 (DmpA) | CUST_3516_PI425702210 | 2.3 |
| Substrate-binding region of ABC-type glycine betaine | CUST_25_PI425702210 | 2.2 |
| Extracellular solute-binding protein, family 1 | CUST_3494_PI425702210 | 2.2 |
| Glycine cleavage system H protein | CUST_4369_PI425702210 | 2.2 |
| Phosphoribosyl-ATP pyrophosphatase | CUST_381_PI425702210 | 2.2 |
| Sodium:dicarboxylate symporter | CUST_2762_PI425702210 | 2.1 |
| Porphyromonas-type peptidyl-arginine deiminase | CUST_2360_PI425702210 | 2.1 |
| Extracellular solute-binding protein, family 3 | CUST_309_PI425702210 | 2.1 |
| Pyrroline-5-carboxylate reductase | CUST_5298_PI425702210 | 2.1 |
| Sulfate adenylyltransferase subunit 1/adenylylsulfate kinase | CUST_875_PI425702210 | 2.1 |
| Yeast 2-isopropylmalate synthase | CUST_4572_PI425702210 | 2.0 |
| Sarcosine oxidase, alpha-subunit, heterotetrameric | CUST_5183_PI425702210 | 2.0 |
| Carbohydrate transport and metabolism | | |
| Glyceraldehyde-3-phosphate dehydrogenase, type I | CUST_3843_PI425702210 | 2.8 |
| Glyceraldehyde 3-phosphate dehydrogenase | CUST_6601_PI425702210 | 2.7 |
| Major facilitator superfamily MFS 1 | CUST_1647_PI425702210 | 2.5 |
| Alpha amylase, catalytic region | CUST_2524_PI425702210 | 2.2 |

**Table 5** (continued)

| COG and gene description | Probe name | FC |
|---|---|---|
| Phosphoenolpyruvate synthase (PpsA) | CUST_6128_PI425702210 | 2.1 |
| Transketolase subunit A | CUST_2708_PI425702210 | 2.0 |
| General substrate transporter | CUST_5028_PI425702210 | 2.0 |
| Coenzyme transport and metabolism | | |
| Molybdopterin synthase subunit (MoaE) | CUST_2137_PI425702210 | 2.6 |
| D-Isomer specific 2-hydroxyacid dehydrogenase, NAD-binding | CUST_2972_PI425702210 | 2.5 |
| Dihydropteroate synthase | CUST_770_PI425702210 | 2.4 |
| 5-Formyltetrahydrofolate cyclo-ligase | CUST_5416_PI425702210 | 2.4 |
| Adenosylhomocysteinase | CUST_5258_PI425702210 | 2.4 |
| Pyridoxamine 5′-phosphate oxidase-like, FMN-binding | CUST_4308_PI425702210 | 2.3 |
| Pyridoxamine 5′-phosphate oxidase-like, FMN-binding | CUST_5664_PI425702210 | 2.3 |
| Dimethylmenaquinone methyltransferase | CUST_2973_PI425702210[a] | 2.2 |
| Ubiquinone biosynthesis protein (P) | CUST_5086_PI425702210 | 2.1 |
| Energy production and conversion | | |
| Cytochrome c oxidase cbb3-type, subunit I | CUST_1822_PI425702210 | 3.3 |
| 3-Oxoglutarate dehydrogenase E1 component | CUST_1609_PI425702210 | 3.0 |
| Aldehyde dehydrogenase (P) | CUST_6818_PI425702210 | 3.0 |
| Cytochrome C oxidase, mono-heme subunit/FixO | CUST_1821_PI425702210 | 2.9 |
| Aldehyde dehydrogenase (NAD+) | CUST_4967_PI425702210 | 2.9 |
| 2-Oxo-acid dehydrogenase E1 component homodimeric type | CUST_461_PI425702210 | 2.8 |
| Luciferase-like | CUST_3247_PI425702210 | 2.8 |
| Aldo/keto reductase | CUST_3715_PI425702210 | 2.8 |
| 2-Oxoglutarate dehydrogenase E2 component | CUST_1610_PI425702210 | 2.8 |
| Quinone oxidoreductase (P) | CUST_6610_PI425702210 | 2.8 |
| Citrate synthase | CUST_1604_PI425702210 | 2.7 |
| Fumarate reductase/succinate dehydrogenase flavoprotein-like | CUST_2732_PI425702210 | 2.7 |
| Succinate dehydrogenase subunit B | CUST_1608_PI425702210 | 2.7 |
| Dihydrolipoamide dehydrogenase | CUST_1611_PI425702210 | 2.6 |
| Phosphoenolpyruvate carboxykinase (ATP) | CUST_266_PI425702210 | 2.6 |
| NADP oxidoreductase, coenzyme F420-dependent | CUST_2067_PI425702210 | 2.6 |
| Cytochrome o ubiquinol oxidase, subunit III | CUST_6759_PI425702210 | 2.6 |
| Assimilatory nitrate reductase (NADH) alpha-subunit apoprotein | CUST_1774_PI425702210 | 2.6 |
| Cytochrome o ubiquinol oxidase, subunit III | CUST_4624_PI425702210 | 2.6 |
| Cytochrome o ubiquinol oxidase subunit II | CUST_4626_PI425702210 | 2.5 |
| Succinate dehydrogenase subunit A | CUST_1607_PI425702210 | 2.5 |
| Succinate dehydrogenase subunit D | CUST_1606_PI425702210 | 2.5 |
| 4Fe-4S ferredoxin, iron-sulfur binding | CUST_1813_PI425702210 | 2.5 |
| Succinate dehydrogenase subunit C | CUST_1605_PI425702210 | 2.5 |
| Aldehyde dehydrogenase | CUST_2354_PI425702210 | 2.4 |
| 4Fe-4S ferredoxin, iron-sulfur binding | CUST_2519_PI425702210 | 2.4 |
| ATP synthase F0, subunit B | CUST_5705_PI425702210 | 2.4 |
| Ubiquinol oxidase, subunit II (CyoA) | CUST_6760_PI425702210 | 2.4 |
| Succinyl-CoA synthetase (ADP-forming) beta-subunit | CUST_1612_PI425702210 | 2.4 |
| Aconitate hydratase 2 (AcnB) | CUST_6510_PI425702210 | 2.4 |

**Table 5** (continued)

| COG and gene description | Probe name | FC |
|---|---|---|
| Cytochrome c2 precursor | CUST_6541_PI425702210 | 2.4 |
| Cytochrome c, class I | CUST_3535_PI425702210 | 2.4 |
| 4Fe-4S ferredoxin, iron-sulfur binding | CUST_1130_PI425702210 | 2.3 |
| Aldehyde dehydrogenase (acceptor) | CUST_5510_PI425702210 | 2.3 |
| Succinate semialdehyde dehydrogenase | CUST_185_PI425702210 | 2.2 |
| NADPH-glutathione reductase | CUST_2916_PI425702210 | 2.2 |
| Aldehyde dehydrogenase (acceptor) | CUST_2117_PI425702210 | 2.2 |
| Oxidoreductase FAD-binding region | CUST_5193_PI425702210 | 2.2 |
| ATP synthase F1, alpha-subunit | CUST_5703_PI425702210 | 2.2 |
| Delta-1-pyrroline-5-carboxylate dehydrogenase/L-proline dehydrogenase | CUST_450_PI425702210 | 2.2 |
| Ferredoxin | CUST_1901_PI425702210 | 2.2 |
| Cytochrome B561 | CUST_3647_PI425702210 | 2.1 |
| Isocitrate dehydrogenase ($NADP^+$) | CUST_3577_PI425702210 | 2.1 |
| NADP-dependent malic enzyme | CUST_5885_PI425702210 | 2.1 |
| FAD-linked oxidase-like protein | CUST_746_PI425702210 | 2.1 |
| Oxidoreductase FAD/NAD(P)-binding | CUST_1182_PI425702210 | 2.0 |
| Cytochrome c, class I | CUST_84_PI425702210 | 2.0 |
| Fe-S cluster assembly scaffold (IscU) | CUST_4589_PI425702210 | 2.0 |
| Electron transfer flavoprotein, alpha-subunit | CUST_5199_PI425702210 | 2.0 |
| Inorganic ion transport and metabolism | | |
| Taurine ABC transporter, periplasmic binding protein | CUST_5396_PI425702210 | 3.2 |
| Taurine ABC transporter, periplasmic binding protein (TauA) | CUST_6882_PI425702210 | 3.0 |
| TonB-dependent receptor | CUST_4986_PI425702210 | 2.7 |
| TonB-dependent copper receptor | CUST_5926_PI425702210 | 2.6 |
| TonB-dependent copper receptor | CUST_594_PI425702210 | 2.5 |
| $K^+$-transporter | CUST_1189_PI425702210 | 2.5 |
| Zinc transporter ZIP | CUST_2776_PI425702210 | 2.4 |
| Ferritin and Dps | CUST_565_PI425702210 | 2.3 |
| FecR (P) | CUST_122_PI425702210 | 2.2 |
| Heme receptor (HasR) | CUST_6466_PI425702210 | 2.2 |
| Periplasmic solute binding protein | CUST_3905_PI425702210 | 2.2 |
| Choline sulfatase | CUST_5818_PI425702210 | 2.0 |
| Nitrite and sulfite reductase 4Fe-4S region | CUST_3096_PI425702210 | 2.0 |
| Intracellular trafficking, secretion and vesicular transport | | |
| Twin-arginine translocation protein (TatA/E) | CUST_380_PI425702210 | 2.1 |
| Lipid transport and metabolism | | |
| Phospholipase D/transphosphatidylase | CUST_1293_PI425702210 | 3.4 |
| Short-chain dehydrogenase/reductase SDR | CUST_4061_PI425702210 | 3.2 |
| 3-Oxoacyl-[acyl-carrier-protein] synthase II | CUST_4016_PI425702210 | 3.0 |
| 3-Oxoacyl-[acyl-carrier-protein] synthase | CUST_6639_PI425702210 | 3.0 |
| Short-chain dehydrogenase/reductase SDR | CUST_300_PI425702210 | 3.0 |
| Acyltransferase 3 | CUST_3089_PI425702210 | 2.7 |
| 3-Oxoacyl-acyl carrier protein synthase II | CUST_4017_PI425702210 | 2.7 |
| Short-chain dehydrogenase/reductase SDR | CUST_900_PI425702210 | 2.6 |

**Table 5** (continued)

| COG and gene description | Probe name | FC |
|---|---|---|
| 3-Oxoacyl-[acyl-carrier-protein] reductase | CUST_4135_PI425702210 | 2.5 |
| Phospholipase D/transphosphatidylase | CUST_5508_PI425702210 | 2.5 |
| Short-chain dehydrogenase/reductase SDR | CUST_4303_PI425702210 | 2.4 |
| Phosphatidylserine decarboxylase | CUST_507_PI425702210 | 2.3 |
| Short-chain dehydrogenase/reductase SDR | CUST_2459_PI425702210 | 2.3 |
| Short-chain dehydrogenase/reductase SDR | CUST_2772_PI425702210 | 2.2 |
| Short-chain dehydrogenase/reductase SDR | CUST_2857_PI425702210 | 2.2 |
| 3-Oxoacyl-[acyl-carrier-protein] synthase I | CUST_4191_PI425702210 | 2.2 |
| 4-Hydroxy-3-methylbut-2-en-1-yl diphosphate synthase | CUST_4579_PI425702210 | 2.1 |
| Acetyl-CoA carboxylase, biotin carboxylase (AccC) | CUST_6932_PI425702210 | 2.1 |
| 3-Oxoacid CoA-transferase | CUST_2070_PI425702210 | 2.1 |
| Phosphate uptake regulator (PhoU) | CUST_5587_PI425702210 | 2.0 |
| 3-Hydroxydecanoyl-[acyl-carrier-protein]dehydratase | CUST_4190_PI425702210 | 2.0 |
| Biotin carboxylase/acetyl-CoA carboxylase carboxyl transferase alpha-subunit | CUST_5615_PI425702210 | 2.0 |
| Nucleotide transport and metabolism | | |
| Xanthine dehydrogenase, molybdenum binding subunit apoprotein | CUST_2101_PI425702210 | 2.9 |
| 6-*O*-Methylguanine DNA methyltransferase family protein (P) | CUST_6025_PI425702210 | 2.4 |
| GMP synthase (glutamine-hydrolyzing) | CUST_4565_PI425702210 | 2.4 |
| TRNA-hydroxylase | CUST_3376_PI425702210 | 2.3 |
| AMP nucleosidase | CUST_4920_PI425702210 | 2.3 |
| Inosine-5′-monophosphate dehydrogenase | CUST_4566_PI425702210 | 2.2 |
| Phosphoribosylglycinamide formyltransferase | CUST_1627_PI425702210 | 2.0 |
| Secondary metabolites biosynthesis, transport and catabolism | | |
| UbiE/COQ5 methyltransferase | CUST_1886_PI425702210 | 2.4 |
| Aa adenylation | CUST_3918_PI425702210 | 2.1 |
| Poorly characterized (8 %) | | |
| Function unknown | | |
| OstA-like protein | CUST_5963_PI425702210 | 3.1 |
| Alkylhydroperoxidase AhpD core | CUST_3185_PI425702210 | 2.9 |
| Outer membrane autotransporter barrel | CUST_2885_PI425702210 | 2.6 |
| OstA-like protein | CUST_856_PI425702210 | 2.4 |
| 3-Demethylubiquinone-9 3-methyltransferase | CUST_4059_PI425702210 | 2.4 |
| Rhs element Vgr protein | CUST_2038_PI425702210 | 2.4 |
| Import inner membrane translocase, subunit Tim44 | CUST_5621_PI425702210 | 2.3 |
| FlhB domain protein | CUST_1570_PI425702210 | 2.3 |
| HesB/YadR/YfhF | CUST_3088_PI425702210 | 2.3 |
| Peptidoglycan-binding (LysM) | CUST_1890_PI425702210 | 2.3 |
| Rhs element Vgr protein | CUST_452_PI425702210 | 2.3 |
| Integral membrane protein | CUST_5228_PI425702210 | 2.1 |
| General function prediction only | | |
| Zinc-containing alcohol dehydrogenase superfamily | CUST_3321_PI425702210 | 3.1 |
| Hydrolase, carbon-nitrogen family | CUST_6748_PI425702210 | 3.0 |
| Amidohydrolase | CUST_721_PI425702210 | 2.9 |
| Formate dehydrogenase gamma subunit | CUST_2689_PI425702210 | 2.9 |

**Table 5** (continued)

| COG and gene description | Probe name | FC |
|---|---|---|
| Sugar fermentation stimulation protein | CUST_4780_PI425702210 | 2.7 |
| Pirin-like | CUST_1622_PI425702210 | 2.6 |
| LamB/YcsF | CUST_1400_PI425702210 | 2.6 |
| Endonuclease/exonuclease/phosphatase | CUST_2527_PI425702210 | 2.6 |
| Lipoprotein (P) | CUST_4850_PI425702210 | 2.6 |
| Lipoprotein (P) | CUST_1438_PI425702210 | 2.5 |
| ThiJ/PfpI | CUST_2237_PI425702210 | 2.5 |
| Phosphatase (KdsC) | CUST_858_PI425702210 | 2.4 |
| ATPases-like | CUST_1038_PI425702210 | 2.4 |
| 4-Oxalocrotonate tautomerase | CUST_3383_PI425702210 | 2.3 |
| Alpha/beta hydrolase fold family | CUST_4181_PI425702210 | 2.2 |
| GCN5-related *N*-acetyltransferase | CUST_580_PI425702210 | 2.1 |
| Hemolysin-type calcium-binding region | CUST_4225_PI425702210 | 2.1 |
| Esterase/lipase/thioesterase | CUST_2230_PI425702210 | 2.0 |
| Aminoglycoside phosphotransferase | CUST_5109_PI425702210 | 2.0 |
| Unclassified (7 %) | | |
| Metallothionein (P) | CUST_3747_PI425702210 | 3.2 |
| Involved in intracellular protein transport (P) | CUST_6753_PI425702210 | 2.7 |
| Lyase (P) | CUST_5234_PI425702210 | 2.6 |
| Fatty acid *cis-trans* isomerase | CUST_3090_PI425702210 | 2.5 |
| Colicin-E3 | CUST_6231_PI425702210 | 2.4 |
| Conserved protein | CUST_6373_PI425702210 | 2.4 |
| Toxin protein (P) | CUST_5927_PI425702210 | 2.4 |
| Aconitase | CUST_3379_PI425702210 | 2.4 |
| 1,4-Alpha-glucan branching enzyme | CUST_2525_PI425702210 | 2.4 |
| Uncharacterized protein family (UPF0153) | CUST_6035_PI425702210 | 2.4 |
| UspA | CUST_3377_PI425702210 | 2.4 |
| Protein (RdxA) | CUST_6299_PI425702210 | 2.3 |
| HTH-type transcriptional activator (AaeR) | CUST_6381_PI425702210 | 2.3 |
| $H^+$-transporting two-sector ATPase, delta (OSCP) subunit | CUST_5704_PI425702210 | 2.3 |
| Enolase | CUST_1118_PI425702210 | 2.3 |
| LOC407663 protein | CUST_6371_PI425702210 | 2.3 |
| Translation elongation factor Tu | CUST_5059_PI425702210 | 2.3 |
| Kelch repeat-containing protein | CUST_2517_PI425702210 | 2.3 |
| Probable AcnD-accessory protein (PrpF) | CUST_6126_PI425702210 | 2.2 |
| NAD(+) kinase | CUST_2187_PI425702210 | 2.2 |
| LemA | CUST_1295_PI425702210 | 2.2 |
| Inner membrane (CreD) | CUST_5171_PI425702210 | 2.1 |
| Phosphonate uptake transporter | CUST_2420_PI425702210 | 2.1 |
| RaxQ | CUST_5965_PI425702210 | 2.1 |
| AMP-dependent synthetase and ligase | CUST_4331_PI425702210 | 2.1 |
| AMP-dependent synthetase and ligase | CUST_4183_PI425702210 | 2.0 |

*FC* fold change between 5 and 15 min exposure time, *P* putative, *Aa* amino acid

[a] Genes that were also differentially expressed over exposure time in the absence of metals

transporters, thiosulfate-binding proteins and taurine catabolism proteins. Similarly, some transmembrane transporters were repressed: ABC transporters (14 genes), general substrate transporters (5 genes), binding-protein-dependent transporters (13 genes), MFS proteins (6 genes), genes encoding the OmpA/MotB system (3 genes), TonB-dependent outer membrane siderophore receptors and drug resistance transporters (2 genes).

Alginate biosynthesis was strongly inhibited, as reflected in the repression of several genes: *algK*, *algJ*, *alg44* and *alg8* belonging to the algD-alg8-alg44-algKEGXLIJFA operon (Kiliç et al. 2010). Alginates are exopolysaccharides known to chelate metals and, hence, can increase bacterial metal tolerance (Poirier et al. 2014). By contrast, Pagès et al. (2007) found an overexpression of alginate biosynthesis in Cd-exposed *P. brassicacearum*.

Pertaining to transcription regulators, we detected a repression of genes belonging to the LysR (11 genes), AsnC (2 genes), GntR (2 genes), TetR (2 genes), IclR, LacI, DeoR and MerR families, as well as of the CusR heavy metal response transcriptional regulator.

Concerning gene overexpression (cluster II), 4MD treatment led to a clear activation of the antioxidative system: overexpression of genes encoding glutathione *S*-transferase, glutathionylspermidine synthetase, spermidine/putrescine ABC transporter, thiol peroxidase (2-Cys peroxiredoxin) and two alkyl hydroperoxide reductases. Nevertheless, genes related to glutathione *S*-transferase and a spermidine/putrescine ABC transporter were also overexpressed in control cells (in the absence of metals), suggesting their constitutive expression under the specific conditions of this experiment. Furthermore, our data revealed an overexpression of TonB-dependent receptors as well as membrane transporters, such as MotA/TolQ/ExbB, MscS, OmpA/MotB and OmpF. Likewise, genes related to chemotaxis were overexpressed: CheZ phosphatase, CheA signal-transducing histidine kinase and the chemotaxis response regulator CheY. On the other hand, genes involved in protein folding (chaperonin 10, chaperonin 60, Hsp70, Hsp90 and PpiC-type peptidyl-prolyl *cis-trans* isomerase) and genes belonging to the Clp protein family (*clpX*, *clpA*, *clpP*, *clpS*, *clpA-B*) were also overexpressed under 4MD treatment. Some members of the Clp protein family are implicated in proteolysis regulation; however, besides being regulators of energy-dependent proteolysis, Clp proteins may also function as molecular chaperones (Li et al. 2012). Certain Clp proteins play a decisive role in determining the destiny of proteins, not only during normal growth but also under conditions of extreme stress (Jain and Bhatt 2013). In *E. coli*, we found an overexpression of genes encoding heat shock proteins (e.g. *clpP*, *hslV-clpQ*, *dnaK*, *ibpA* and *ibpB*) when cells were exposed to the intermediate metal dose (MD) (Gómez-Sagasti et al. 2014). This similar gene expression pattern in both species (*P. fluorescens* and *E. coli*) suggests the importance of chaperone systems in response to the presence of metals. Similarly, succinate dehydrogenase A/B/C/D genes were overexpressed under 4MD treatment. Miller et al. (2009) interpreted the overexpression of succinate dehydrogenase in metal-exposed *P. putida* cells as a response to metal-induced oxidative stress.

In the same way, 18 LSU ribosomal protein genes, 12 SSU ribosomal protein genes and 3 Rpl/Rpm/Rps ribosomal protein genes were overexpressed. Finally, transcription regulators were either overexpressed (i.e. AraC, MarR and LuxR) or repressed (i.e., GntR, LysR, LacI and TetR), suggesting that, under 4MD treatment, *P. fluorescens* cells developed a complex transcriptional modulation over exposure time.

### Genes differentially expressed under more than one polymetallic treatment

Genes differentially expressed under more than one polymetallic treatment (and not differentially expressed under control treatment) were identified, in an attempt to look for potential biomarkers of the effect of exposure time on the early gene expression of Gram-negative bacterial cells exposed to a polymetallic solution. Only one probe was differentially expressed under all three polymetallic treatments (MD/4, MD, 4MD): a gene encoding a GCN5-related *N*-acetyltransferase (Table 6). On the other hand, six genes were differentially expressed under both MD/4 and MD treatments: arginine/ornithine antiporter, IndB protein, $H^+$ transporter ATPase, malate synthase G, P-loop ATPase and YceI protein. In turn, 24 genes were differentially expressed under both MD and 4MD treatments (Table 6), such as, for instance, genes encoding AlgJ protein, alkyl hydroperoxide reductase, arginine/ornithine antiporter, succinate semialdehyde dehydrogenase, sulfatase, taurine transporter, thiosulfate-binding protein, TonB-dependent receptor, transcription regulators (AraC,

**Table 6** *Pseudomonas fluorescens* genes differentially ($p<0.01$) expressed over exposure time under more than one polymetallic treatment

| |
|---|
| Common genes between MD/4, MD and 4MD |
| [CUST_2605_PI425702210] GCN5-related *N*-acetyltransferase |
| Common genes between MD/4 and MD |
| [CUST_4362_PI425702210] Arginine/ornithine antiporter |
| [CUST_6665_PI425702210] IndB protein |
| [CUST_5700_PI425702210] $H^+$-transporting two-sector ATPase,delta/epsilon subunit |
| [CUST_5161_PI425702210] Malate synthase G |
| [CUST_851_PI425702210] P-loop ATPase protein UPF0042 (P) |
| [CUST_5262_PI425702210] YceI-like family protein |
| Common genes between MD/4 and 4MD |
| [CUST_5926_PI425702210] TonB-dependent copper receptor |
| [CUST_759_PI425702210] GrpE protein |
| [CUST_1560_PI425702210] Chemotaxis phosphatase (CheZ) |
| [CUST_760_PI425702210] Heat shock protein (Hsp70) |
| [CUST_4557_PI425702210] PIM1 peptidase. Serine peptidase. MEROPS family S16 |
| [CUST_3573_PI425702210] ATP-dependent Clp protease ATP-binding subunit (ClpA) |
| [CUST_4982_PI425702210] Filamentation induced by cAMP protein Fic |
| [CUST_2459_PI425702210] Short-chain dehydrogenase/reductase SDR |
| [CUST_5713_PI425702210] TRNA modification GTPase (TrmE) |
| [CUST_2101_PI425702210] Xanthine dehydrogenase, molybdenum binding subunit apoprotein |
| [CUST_1835_PI425702210] Glucose 1-dehydrogenase |
| [CUST_2955_PI425702210] Aromatic-ring-hydroxylating dioxygenase, beta-subunit |
| [CUST_3437_PI425702210] Gluconolactonase |
| [CUST_3631_PI425702210] Acetyltransferase, GNAT family |
| [CUST_1274_PI425702210] Outer membrane porin |
| [CUST_6809_PI425702210] Shikimate 5-dehydrogenase |
| [CUST_5427_PI425702210] Glycerol-3-phosphate transporter |
| Common genes between MD and 4MD |
| [CUST_2857_PI425702210] Short-chain dehydrogenase/reductase SDR |
| [CUST_4366_PI425702210] Carbamate kinase |
| [CUST_2905_PI425702210] 4-Hydroxyphenylpyruvate dioxygenase |
| [CUST_4363_PI425702210] Arginine/ornithine antiporter |
| [CUST_6714_PI425702210] Arginine/ornithine antiporter |
| [CUST_1822_PI425702210] Cytochrome C oxidase cbb3-type, subunit I |
| [CUST_1821_PI425702210] Cytochrome C oxidase, mono-heme subunit/FixO |
| [CUST_2250_PI425702210] Transcriptional regulator, AraC family |
| [CUST_3108_PI425702210] TonB-dependent receptor |
| [CUST_2573_PI425702210] Sulfatase |
| [CUST_3601_PI425702210] Outer membrane porin |
| [CUST_185_PI425702210] Succinate semialdehyde dehydrogenase |
| [CUST_252_PI425702210] Binding-protein-dependent transport systems IMC |
| [CUST_1400_PI425702210] LamB/YcsF |
| [CUST_4511_PI425702210] Transcriptional regulator, DeoR family |
| [CUST_5621_PI425702210] Import inner membrane translocase, subunit Tim44 |
| [CUST_1895_PI425702210] Argininosuccinate synthase |
| [CUST_5396_PI425702210] Taurine ABC transporter, periplasmic binding protein |

**Table 6** (continued)

| |
|---|
| [CUST_949_PI425702210] Alginate biosynthesis protein (AlgJ) |
| [CUST_5346_PI425702210] Binding-protein-dependent transport systems IMC |
| [CUST_1660_PI425702210] Phospholipid/glycerol acyltransferase |
| [CUST_192_PI425702210] Thiosulfate-binding protein |
| [CUST_2648_PI425702210] Secretion protein (HlyD) |
| [CUST_2913_PI425702210] Alkyl hydroperoxide reductase/Thiol specific antioxidant/Mal allergen |

DeoR), etc. Unexpectedly, 17 genes were differentially expressed under both MD/4 and 4MD treatments; MD/4 and MD treatments shared only 6 differentially expressed genes, despite being closer in terms of heavy metal concentration (Fig. 2).

## Comparison of gene expression patterns between *P. fluorescens* and *E. coli*

Despite being subjected to the same experimental conditions, transcriptional patterns in *P. fluorescens* were different to those observed in *E. coli* in a previous study

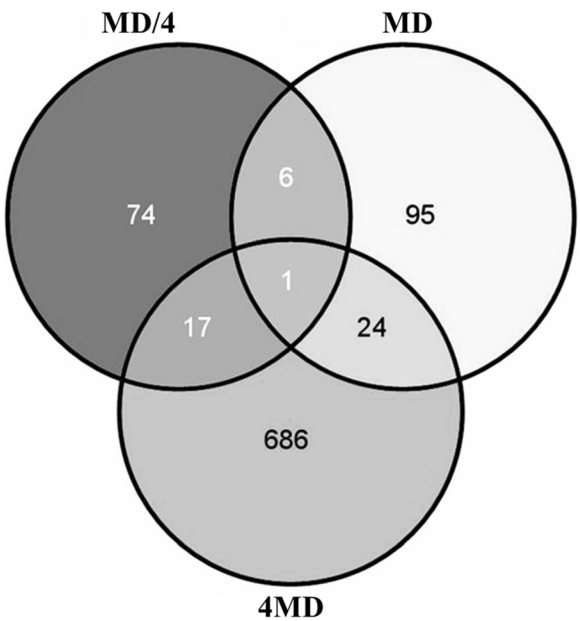


**Fig. 2** Venn diagram showing the overlap of genes differentially expressed over exposure time under the different polymetallic treatments (*MD/4*, *MD*, *4MD*). MD (metal dose)=10 μM Ag(I), 10 μM Pb(II), 10 μM Cd(II), 10 μM Cu(II), 500 μM Ni(II) and 300 μM Zn(II). *MD/4* fourfold lower metal dose, *4MD* fourfold higher metal dose. Venn diagrams use overlapping circles to visually represent the commonalities amongst sets of information. The number of differentially expressed genes shared by different polymetallic treatments is displayed in the overlapping circles

(Gómez-Sagasti et al. 2014). In fact, over exposure time, *P. fluorescens* regulated a greater number of genes than *E. coli* at each metal dose (Fig. 3). At MD/4, both *P. fluorescens* and *E. coli* overexpressed metal-specific transporters, in order to cope with metal stress (Fig. 3). On the other hand, relevant cellular response mechanisms observed over exposure time in *P. fluorescens* and *E. coli* cells appear to occur in different cellular locations: the expression of genes involved in chemotaxis and membrane permeability (i.e. stimuli sensors and efflux pumps) was fine-tuned in *P. fluorescens* (i.e. some genes were upregulated while others were downregulated) whereas in *E. coli*, metal exposure led to the regulation of genes related to reactive oxygen species (ROS) and sulfur homeostasis in the cell interior (i.e. superoxide dismutase SodA, Fe-S cluster assembly scaffold and synthesis of cysteine).

Under MD treatment, heavy metals increased the generation of ROS, overwhelming the antioxidant defences of *P. fluorescens* and *E. coli* cells and then resulting in oxidative stress. As a consequence, genes encoding ROS scavengers were overexpressed in both species (Fig. 3). On the other hand, *P. fluorescens* cells showed an overexpression of genes related to sulfur transport and iron acquisition over exposure time. This might be understood as a tolerance mechanism probably designed to promote the chelation of free metals and mitigate metal-induced iron deficiencies (Teitzel et al. 2006). Nonetheless, we observed toxicity symptoms in *P. fluorescens* cells due to the disruption of chemotaxis sensors, amino acid metabolism and DNA repairment. Conversely, *E. coli* cells overexpressed genes encoding heat shock proteins, in order to protect and recycle peptides/macromolecules in the face of metal stress. At this MD dose, in the Gram-positive bacterium *Bacillus subtilis*, CDF (Cation Diffusion Facilitators) family members were responsible for cell protection against sudden exposure to Zn(II), Cu, Co(II) and Ni(II) (Moore et al. 2005). In addition, pathways related to

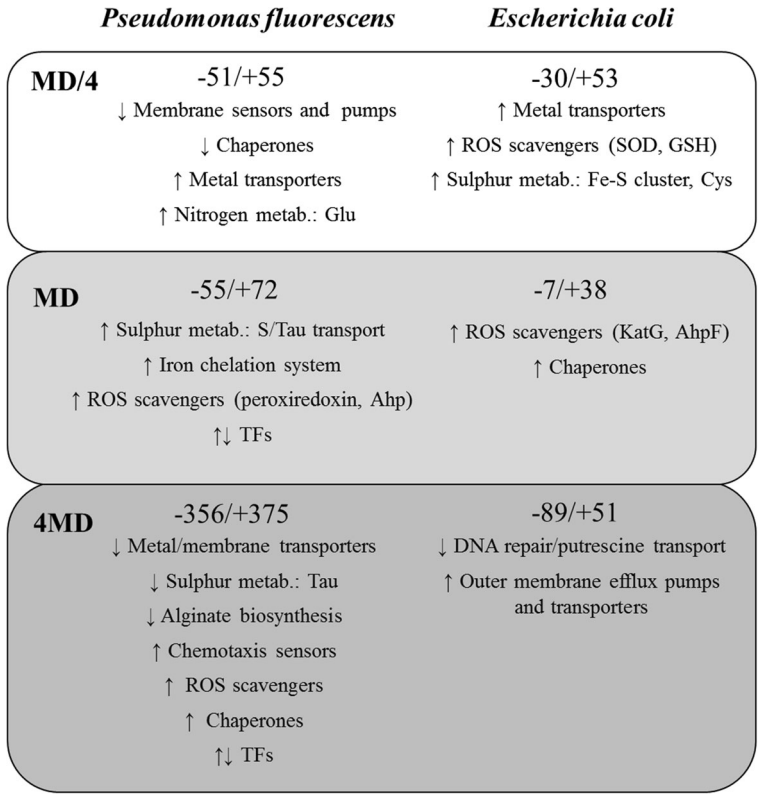


**Fig. 3** Comparison of *P. fluorescens* and *E. coli* gene expression patterns under MD/4, MD and 4MD treatments. − number of repressed genes, + number of overexpressed genes. *ROS* reactive oxygen species, *SOD* superoxide dismutase, *GSH* glutathione, *Glu* glutamine, *Cys* cysteine, *Tau* taurine, *TFs* transcription factors, ↑ overexpression, ↓ repression

cysteine were strongly induced by metal stress in *B. subtilis* (Moore et al. 2005).

Finally, 4MD treatment appeared to cause a somewhat greater disturbance to *E. coli* cells over exposure time, as compared to *P. fluorescens* which activated important protection mechanisms in response to metal exposure: thus, *P. fluorescens* cells maintained the transcription of ROS scavengers and overexpressed chaperone-coding genes, as well as genes related with ribosome formation (Fig. 3); instead, *E. coli* cells did not show an early and coordinated transcriptional response in response to metal exposure. In fact, according to the growth curves obtained in the presence of metals, *E. coli* growth was more sensitive to the metal treatments used here than *P. fluorescens*.

The abovementioned transcriptional differences between both Gram-negative bacteria might be, at least in part, due to their adaptation to different environmental conditions: *P. fluorescens* is a common inhabitant of the soil and rhizosphere environment (Varivarn et al. 2013) and harbours a large panel of metal resistance and tolerance mechanisms (Aguilar-Barajas et al. 2010); on the contrary, *E. coli* is most commonly found as a commensal of the intestinal tract of warm-blooded animals (Tenaillon et al. 2010).

## Validation of microarray results by RT-qPCR

Amplification efficiencies of primer pairs were between 93 and 105 % (that is to say, acceptable for quantification by RT-qPCR). Primer specificity was confirmed by melting curves analysis, which showed the amplification of a single PCR product. For most of the selected genes, Ct values were close to 30. If we consider Ct values around 35 as negative, our selected genes could be classified as low-expression genes.

Regarding the reference genes (*fabD*, *rsd*/*algQ*, *pyr*, *16S*), the *geNorm* analysis indicated that *rsd*/*algQ* (regulator of sigma factor RpoD) and *16S* (16S rRNA) genes showed a high expression stability, as reflected by the stability measure M (Vandesompele et al. 2002; Gómez-Sagasti et al. 2014). Similarly, *NormFinder* analysis showed *rsd*/*algQ* gene (SD=0.0251) as an optimal reference gene for the quantification of transcriptional responses by RT-qPCR. Moreover, unlike *16S* gene, Ct values for *rsd*/*algQ* were within the range of the values

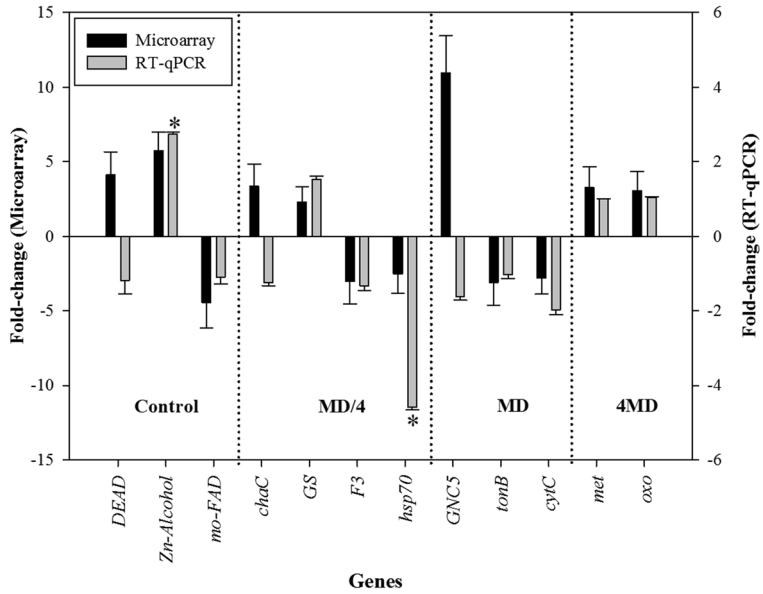


**Fig. 4** Validation of microarray results by RT-qPCR. *Rectangles* represent expression fold changes of selected genes (*DEAD*, *Zn-Alcohol* and *mo-FAD* for control treatment; *chaC*, *GS*, *F3* and *hps70* for MD/4 treatment; *GNC5*, *tonB* and *cytC* for MD treatment; *met* and *oxo* for 4MD treatment) as measured by microarray and RT-qPCR. *Bars*, standard deviations ($n$=2)

observed for the selected genes. In consequence, *rsd*/*algQ* was used here as a stable and accurate reference gene.

Out of the 16 genes selected for validation, 4 genes (*lysR*, *chem*, *ATPase*, *PFKB*) were not detected by the designed primers (Table 1). Out of the remaining 12 genes, 9 genes showed the same response trend (over-expression or repression) in microarray vs. RT-qPCR analysis (Fig. 4). But, according to the RT-qPCR analysis, only 2 genes (*hsp70* and *Zn-Alcohol*) were differentially expressed over exposure time (5 vs. 15 min; ANOVA $p<0.05$) (Fig. 4).

Under both techniques, the obtained fold changes for each gene were moderately correlated (Pearson's, $r$= 0.822) (Fig. 5). Correlation values between microarray and RT-qPCR results can vary between 0.48 and 0.94 (Morey et al. 2006). Morey et al. (2006) indicated that criteria for the determination of an acceptable validation of microarray results by RT-qPCR are seldom defined. Then, since nine of the selected genes showed the same response trend under RT-qPCR and microarray analysis, we considered our microarray results validated by RT-qPCR.

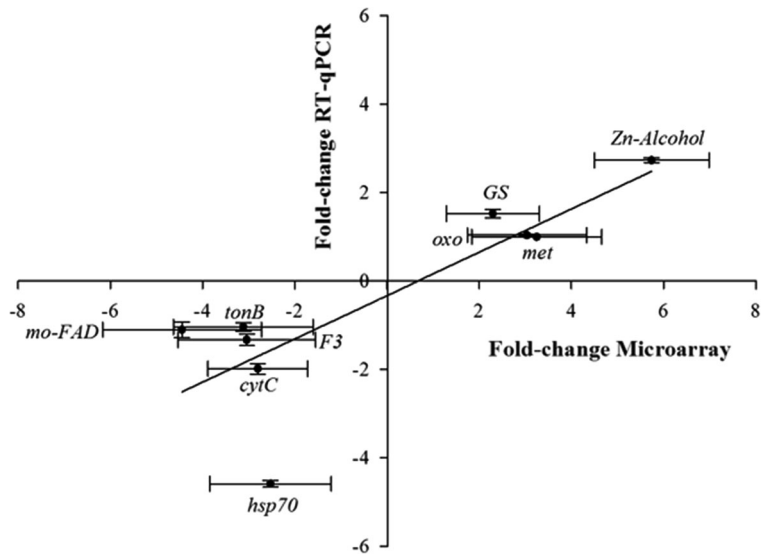


**Fig. 5** Correlation plot between fold changes of genes differentially expressed in the RT-qPCR analysis (*Zn-Alcohol*, *mo-FAD*, *GS*, *F3*, *hsp70*, *tonB*, *cytC*, *met*, *oxo*) and their corresponding fold-change values in the microarray analysis. $r$, Pearson's $r$. *Horizontal bars*, standard deviations ($n$=2) for microarray data; *vertical bars*, standard deviations ($n$=2) for RT-qPCR data

## Conclusions

We studied the temporal changes in the early gene expression of *P. fluorescens* exposed to three doses of a polymetallic solution over two exposure times (5 and 15 min). In the absence of metals, a lower number of genes, compared to metal treatments, were differentially expressed over exposure time: genes encoding chaperones, secretion proteins, membrane structural proteins, redox balance proteins and transcription regulators were differentially expressed. At the lowest heavy metal dose (MD/4), we observed the repression of TonB-dependent copper receptor, the Hsp70 protein folding system, MATE and MFS drug resistance transporters, and histidine kinases, together with the overexpression of metal transport (ChaC and CopC), chemotaxis and glutamine synthetase genes. At the intermediate dose (MD), several amino acid transporters, a stimuli receptor (CheY), a TonB-dependent receptor and the *mutT* DNA repair gene were repressed; by contrast, an overexpression of genes associated with the antioxidative stress system and the transport of chelates and sulfur was observed. Finally, at the highest dose, a repression of genes encoding metal ion transporters, drug resistance, and alginate biosynthesis was found, together with an overexpression of genes encoding antioxidative proteins, membrane transporters, ribosomal proteins, chaperones and proteases. Over exposure time, *P. fluorescens* cells showed a complex cellular response when exposed to a polymetallic solution, involving mechanisms related with chemotaxis, signal transmission, membrane transport, cellular redox state, and regulation of transcription and ribosomal activity. In contrast to gene expression patterns shown by *E. coli* cells exposed to the same polymetallic treatments under the same experimental conditions, *P. fluorescens* cells regulated the expression of genes associated with signalling and chemotaxis, alginate biosynthesis, transcription regulation and ribosomal subunit formation. As reflected by their growth curves, *P. fluorescens* appears to be more tolerant to early metal exposure than *E. coli*, possibly due to the regulation of membrane sensing and permeability, as well as the maintenance of antioxidant and chaperone systems at increasing metal doses. Finally, a gene encoding a GCN5-related *N*-acetyltransferase was differentially expressed under all three polymetallic treatments and, thus, could be a potential biomarker of the effect of exposure time on the early gene expression of Gram-negative bacteria exposed to a polymetallic solution; in any case, much further research is needed to ascertain the potential of this gene as biomarker of metal exposure.

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