Summary of the PhD thesis

Metal-inducible promoters of *Synechocystis* sp. 6803 and their use for whole-cell bioreporter development

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INTRODUCTION

Metal* pollution is a quickly growing problem for the aquatic and terrestrial ecosystems. Organisms must tightly control intracellular metal ion levels to avoid toxicity. Toxicity is a result of excessive accumulation of essential metal ions, or a consequence of over-accumulation of metal ions with no biological function. There are primary effects caused by the interactions between metal ions and the cellular components, and there are also secondary effects arising from the displacement of metal ions from biological molecules or from the metal ion-mediated oxidative stress resulting in widespread damage to membranes, proteins and DNA and impairment of enzyme function. Not much is known about the transcriptional responses elicited by excess amount of metal ions in prokaryotes. For our model species *Synechocystis* sp. PCC 6803, only a single DNA microarray study, investigating Cd^{2+}, Zn^{2+} and Fe^{2+}-induced modification in gene expression was published to date [Houot et al. (2007) *BMC Genomics* 8: 350]. There are specific stress genes, induced in response to a particular type of stress, or general stress genes, induced by various stress factors. Many genes for shock proteins (such as *hspA*) and proteases belong to the last category, their expression being induced by oxidative, hyperosmotic, heat, salt, UV-B, and light stress (but not by cold stress).

All organisms possess resistance mechanisms for protection against the excess of metal ions, encoded on plasmid or chromosomal genes. They are believed to have arisen soon after life began, in a biosphere polluted by volcanic activities and other natural geological sources [Silver and Phung (1996) *Annu Rev Microbiol* 50: 753]. The main mechanisms of resistance are: efflux ‘pumping’ of the toxic ions that enter the cell, enzymatic detoxification that converts more toxic

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* For simplicity, throughout this summary, the term metal refers to both metal and semimetal

to less toxic or less available metal ion species, and intracellular sequestration of the toxic metal ions. These processes are usually regulated by metalloregulatory proteins of either the MerR or ArsR/SmtB families.

Analysis of the fully sequenced *Synechocystis* genome [Kaneko et al. (1996) *DNA Res* 3: 10] led to the identification of 11 clustered chromosomal ORFs that encodes homologs of metal transport proteins. The region is organized into six putative transcriptional units: *(i*) the *nrsBACD* operon induced by Ni^{2+} and Co^{2+} and regulated by the upstream *nrsSR* operon products [García-Dominguez et al. (2000) *J Bacteriol* 182: 1507; López-Maury et al. (2002) *Mol Microbiol* 43: 247], (ii) *ziaA,* induced by Zn^{2+}, encoding a putative Zn^{2+} efflux P_1-type ATPases and regulated by the product of the preceding ORF, *ziaR* [Thelwell et al. (1998) *PNAS* 95: 10728], and (iii) *coaT*, induced by Co^{2+} and Zn^{2+}, encoding a putative Co^{2+} translocating P_1-type ATPase under the regulation of the upstream *coaR* product [Rutherford et al. (1999) *J Biol Chem* 274: 25827; García-Dominguez (2000) *J Bacteriol* 182: 1507]. The resistance to arsenic salts in *Synechocystis* is encoded by the *arsBHC* operon. The operon is induced by As^{5+}/As^{3+}/Sb^{3+} and its expression is under the regulation of the ArsR repressor protein. Besides CoaT and ZiaA, *Synechocystis* has two more P_1-type ATPases, CtaA and PacS that are putative copper cation transporters localized in the plasma membrane and thylakoid membrane, respectively [Tottey et al. (2001) *J Biol Chem* 276: 19999]. Atx1 is a metallochaperone which interacts with the amino-terminal domains of CtaA and PacS and might play a role in chaperoning Cu^{2+} en route to the thylakoid [Borelly et al. (2004) *Biochem J* 378: 293; Tottey et al. (2002) *J Biol Chem* 277: 5490].

In the *Synechocystis* genome there are two ORFs that encode members of the chromate ion transporter (CHR) superfamily [Díaz-Pérez et al. (2007) *FEBS J* 274: 6215]: *slr5038*, located on the pSYSM plasmid, and the chromosomally encoded *chrA*. The function has been elucidated only for two members of this superfamily. They are membrane proteins that pump out chromate from the cytoplasm using the proton motive force, conferring in this way chromate resistance [Alvarez et al.

(1999) *J Bact* 181: 7398; Pimentel (2002) *FEMS Microbiol Lett* 212: 249]. Another member of this superfamily, SrpC from *Synechococcus* sp. PCC 7942, is encoded on the plasmid pANL that was shown to be involved in cell adaptation to sulfur starvation. The *srpC* disruption mutant showed an increase, rather than a decrease, in chromate resistance, when grown in low sulfate medium [Nicholson and Laudenbach (1995) *J Bact* 117: 2143]. A hypothesis was formulated that CHR proteins perform chromate/sulfate antiport: they change the intracellular accumulated chromate for sulfate from the growth environment; when the sulfate concentration in the growth environment is occasionally lower than the chromate concentration, the antiporter works as a chromate uptake system, therefore explaining the chromate resistance phenotype of the *srpC* disruption mutant [Nies (1998) *J Bact* 180: 5799].

In the last two decades, bacterial resistance mechanisms against various metal ions have been used to construct whole-cell bioreporters. These are genetically modified living bacteria, which express molecular fusions of regulatory circuits operated by metal ions with reporter genes encoding easily detectable proteins. Hence, they are able to sense the metal ions in their environment, representing an alternative to traditional analytical chemical methods. Their greatest advantage is the ability to detect the bioavailable fraction (rather than total concentration) of an analyte, allowing for more accurate assessment of polluted sites. Photoautotrophic cyanobacteria represent an advantage over the use of heterotrophic microorganisms because they can grow on low-cost media and require little maintenance. Because *Synechocystis* is naturally transformable and its full genomic sequence is available, it represents a suitable organism for whole-cell bioreporter construction.

OBJECTIVES

The main goals of our studies were to

I. Investigate the changes in transcript level for genes that were demonstrated or suggested to be involved in metal ion transport in the cyanobacterium *Synechocystis*, following a short exposure to biologically relevant concentrations of the following metal ions: Co^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+}, Cr^{3+}, Cr^{6+}, As^{3+} and As^{5+}.

II. Characterize the activity of two whole-cell bioluminescent reporters that were previously generated in our laboratory by fusing the Co^{2+}/Zn^{2+} inducible $O/P_{\boldsymbol{coaT}}$ (the operator-promoter region of the *coaT*) or the Ni^{2+}/Co^{2+} inducible $O/P_{\boldsymbol{nrsBACD}}$, with the promotorless *luxAB* reporter genes, and to test their applicability to environmental samples. Generate an arsenic bioreporter on the same principle using the As^{3+}/As^{5+}/Sb^{3+}-inducible $O/P_{\boldsymbol{arsBHC}}$.

III. Investigate the function and regulation of *slr5038* gene that encodes a putative chromate transporter, and was shown by us to be induced by As^{3+} and also by Cr^{6+} when the cells were grown in low sulfate medium.

IV. Find the concentration ranges for Cd^{2+}, Ni^{2+}, As^{3+}, Zn^{2+}, Co^{2+}, and Cu^{2+} in which *Synechocystis* cells respond specifically to the ion excess, as well as the concentration ranges in which general and oxidative stress responses occur.

METHODS

Growth conditions and metal salt treatment

The Synechocystis sp. PCC 6803 wild-type and its mutant derivatives were grown in BG-11 medium [Rippka et al. (1979) *J Gen Microbiol* 111: 1] supplemented with 20 mM HEPES (pH 7.5). The cell cultures were maintained in an incubator set at the following parameters: 30 °C temperature, 120 rpm rotation speed, 3% CO_2-enriched atmosphere, 40 µmol photons m^{-2} s^{-1}. When appropriate, antibiotics were included in the medium at final concentrations of 25 µg ml^{-1} spectinomycin, 5 µg ml^{-1} chloramphenicol or 80 µg ml^{-1} kanamycin. The treatments were carried out in BG-11 medium supplemented with $ZnSO_4$, $CdCl_2$, $NiCl_2$, $CoCl_2$, $NaAsO_2$, KH_2AsO_4, $CuSO_4$, $Cr_2(SO_4)_3$ or Na_2CrO_4.

Growth inhibition caused by excess metal ions

The growth of cyanobacterial cultures was quantified by measuring the optical density at 720 nm for a period of 3 to 4 days. Two inhibition parameters were determined for each metal ion: minimal inhibitory concentration (IC_{min}) and maximal inhibitory concentration (IC_{max}). The IC_{min} refers to the lowest tested concentration leading to growth inhibition, whereas IC_{max} refers to the highest tested concentration where no further growth was observed.

Nucleic acid extraction and quantitative real-time PCR analysis

Total RNA was isolated from *Synechocystis* cultures by the hot phenol method [Mohamed and Jansson (1989) *Plant Mol Biol* 13: 693]. Genomic DNA contamination was reduced by treatment with DNase according to the protocol provided by Turbo DNA-free™ kit (Ambion). The reverse transcription and the quantitative real-time PCR were performed following the instructions provided by High Cappacity cDNA Reverse Transcription Kit and Power SYBR Green PCR

Master Mix (Applied Biosystems). Oligonucleotides were designed using Primer Express 2.0 software (Applied Biosystems). Minus RT controls (without reverse transcriptase) were included for each cDNA sample (to detect genomic DNA contamination). The relative changes in gene expression were calculated using delta-delta C_T method [Applied Biosystems (2001) User Bulletin #2: 11] and were normalized to the expression of the RNase P subunit B-encoding *rnpB* gene as internal standard.

Generation of bioreporters

Three bioluminescent bioreporters, *nrsLux*, *coaLux,* and *ziaLux* were previously generated in our laboratory [Peca et al. (2008) *FEMS Microbiol Lett* 289: 258]. For generation of the *arsLux* bioreporter, a sequence upstream of the *arsBHC* coding region containing the O/P_{arsBHC} and the 5' end of *sll0914* was ligated upstream of the promoterless *luxAB* luciferase genes, in the vector pND6luxAB. The constructs were used for the transformation of a *Synechocystis* strain harboring the *luxCDE* luciferase substrate genes along with a spectinomycin resistance casette. The constructs were integrated into the chromosome, along with a chloramphenicol resistance cassette, via homologous recombination in the neutral site *ssl0410*. A bioreporter that contains a truncated version of *ziaR* ($ziaLux_T$) was also generated. The growth rates of the bioreporters were comparable with those of the wild type.

Bioluminescence assay

Metal salt treatments were carried out in 96-well black microtiter plates in a volume of 300 µL per well. The plates were covered with needle-punctured transparent foil and incubated for 3 h, or 18 h in the case of *arsLux* reporter strain, in light (40 photons µmol m^{-2} s^{-1}) or darkness. Assays were performed in quadruplicate. Luminescence intensity was determined with a Top Count NXT luminometer (Packard Instruments) and was expressed as counts per second (cps). The relative luminescence induction was calculated by dividing the mean

luminescence signal of a treated sample by the mean luminescence signal of the untreated sample. The half maximal inhibitory concentration (IC_{50}) refers to the concentration of an interfering metal ion that produce 50% inhibition of the bioluminescent response.

Acidic extraction of environmental sample

The soil-like material used for this study consists of a mixture of different chemical and oil industry wastes from Almásfüzitő bauxite residue disposal area in NE Hungary. Samples collected in triplicate from the composting piles were dried and passed through a 2-mm sieve. To assess the exchangeable, acid-soluble fractions of Ni^{2+}, Co^{2+} and Zn^{2+}, one-step acetic acid extraction of the material was carried out according to [Bódog et al. (1996) *Int J Environ An Ch* 66:79]. Aliquots of 1.0 g soil were mixed with 40 ml 0.11 M CH_3COOH. The suspension was shaken for 16 h at room temperature and then centrifuged for 15 min at 1500 *g*. A volume of 0.04 ml HNO_3 was added to the supernatant diluted with water to 50 ml. The metal content was determined using an atomic absorption spectrometer (Perkin-Elmer model 3110). Each soil sample was analyzed in triplicate. For the bioluminescence measurements, the pH of the CH_3COOH-extracted sample was adjusted to 7.5 with 1M Tris-HCl pH 8.0.

RESULTS

I. Searching for potential promoters for bioreporter construction, we selected ORFs that encode for proteins which have been demonstrated or suggested to be involved in metal ion transport in the cyanobacterium *Synechocystis*, as follows: (i) genes encoding P_1-type ATPases that transport Zn^{2+} (*ziaA*), Co^{2+} (*coaT*) and Cu^{2+} (*ctaA, pacS*), (ii) *atx1* that encodes a copper chaperone, (iii) *slr5038* (designated by us as *artT*) and *chrA* that encode members of the chromate ion transporter (CHR) superfamily, (iv) *nrsB* whose protein product is a putative Ni^{2+} efflux transporter, and (v) *arsB,* encoding a putative As^{3+} exporter. We investigated the induction pattern for these genes by quantitative real-time PCR, upon incubation with concentrations falling between IC_{min} and IC_{max} of the following metal ions: Co^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+}, Cr^{3+}, Cr^{6+}, As^{3+} and As^{5+}. We found that a pronounced gene induction generally occurred within 15 min of exposure, and the expression pattern did not change significantly for the next 45 min. The characteristics of *arsB*, *nrsB*, *coaT* and *ziaA* gene induction are summarized below:

Gene name	Metal ion	Peak level of induction (approximate fold induction)	Interval of concentration corresponding to peak induction
arsB	As^{3+}	1000	500–3500 µM
	As^{5+}	80	2–47 mM
nrsB	Ni^{2+}	700	1–27 µM
	Co^{2+}	5	4–32 µM
coaT	Zn^{2+}	300	32 µM
	Co^{2+}	20	2–32 µM
ziaA	Zn^{2+}	45	2–16 µM
	Cd^{2+}	13	2–16 µM

We found that besides the known Zn^{2+}-triggered induction of the *ziaA* gene, Cd^{2+} is also a strong inducer. Since P_1-type ATP-ases commonly have high specificity for metal ions which they transport [Liu et al. (2002) *J Bacteriol* 184: 5027], we think it is not likely that Cd^{2+} is a gratuitous inducer, but rather it is transported by the ZiaA ATP-ase. The expression of *ziaA* is regulated by ZiaR that belongs to the ArsR/SmtB family of metalloregulatory proteins. Members of the ArsR/SmtB family are repressors that bind as homodimers to the operator–promoter (O/P) sequences of genes mostly involved in transport of metal ions. Metal ion binding induces a conformational change that decrease the affinity of the repressor to the O/P sequence and leads to the release of the metal-bound repressor and the expression of the metal ion transporter [Helmann (2007) Molecular Microbiology of heavy metals*, Springer-Verlag*: 38]. Recent crystallographic studies found that a homolog of ZiaR from the same protein family, namely CadC from *Staphylococcus aureus,* has two metal binding sites, both able to bind Zn^{2+} and Cd^{2+} [Kandegedara et al. (2009) *J Biol Chem* 284: 14958]. One of the regulatory sites (site 1) is composed of four cysteine residues C7, C11, Cys58, and Cys60 [Sun et al. (2001) *J Biol Chem* 276: 14955]. The other regulatory site (site 2) is formed at the dimerization interface and is composed of D^{101} and H^{103} from one monomer and H^{114} and E^{117} from the other monomer [Ye et al. (2005) *J Bacteriol* 187: 4214]. All 4 amino acid residues of site 2 are conserved in *Synechocystis*, together with 3 out of 4 cysteine residues from site 1, making it likely that ZiaR also binds Cd^{2+}, in addition to Zn^{2+}. Therefore, it is possible that, besides Zn^{2+}, ZiaA also exports Cd^{2+}. No cadmium resistance system was yet discovered in *Synechocystis*.

The transcripts of the *ctaA*, *pacS* and *atx1,* all supposed to play a role in copper transport and chaperoning, did not significantly changed during short time incubation with concentrations of Cu^{2+} that belong to the interval (IC_{min}-IC_{max}). Therefore, the expression of *pacS* seems to be regulated differently than that of its homolog gene from *Synechococcus,* whose transcript and protein product levels

were specifically increased upon addition of copper to the growth medium [Kanamaru et al. (1994) *Mol Microbiol* 184: 5027].

II. We have characterized, optimized and shown the potential applications of two whole-cell bioluminescent reporters previously constructed in our laboratory, namely *coaLux* and *nrsLux*. Both the *coaLux* and *nrsLux* strains showed a dose-dependent response to the metal salts added to the culture medium. The detection range of *coaLux* bioreporter was 0.3–6.4 µM for Co^{2+} and 1–3.2 µM for Zn^{2+}. The shape of the concentration-dependent luminescence response for Zn^{2+} and the detection range are similar to those obtained with the cyanobacterial sensor based on *Synechococcus smt-luxCDABE* transcriptional fusion [Erbe et al. (1996) *J Ind Microbiol* 17: 80]. The applicability of the *coaLux* bioreporter for Zn^{2+} detection has been tested using a polluted soil-like sample material collected from a composted mixture of different chemical and oil industry wastes. The *coaLux* reporter strain detected about 92 % of the concentration of zinc determined by atomic absorption spectrometry.

The detection range of *nrsLux* bioreporter was 0.2–8 µM Ni^{2+}. Since zinc and nickel pollution coexist in many sites, the performance of the *nrsLux* reporter strain in mixed samples was tested using Ni^{2+} and Zn^{2+} salt standards. We found that the half maximal inhibitory concentration of Zn^{2+} (IC_{50}) on the Ni^{2+} induced bioluminescence response is about 6 µM. The detection range of *nrsLux* matches the upper nickel concentration limit admitted in the drinking water specified by WHO's Guidelines for Drinking-water Quality (0.07 mg/L = 1.19 µM). Therefore the *nrsLux* bioreporter presented here is potentially useful for Ni^{2+} detection in drinking water, with the limitation that the accompanying zinc concentration is relatively low. Since zinc concentration in tap water is seldom above 0.15 µM, i.e. about 70 times less than IC_{50} for Zn^{2+}, this is not a serious drawback. The luminescence response of the *coaLux* and *nrsLux* bioreporters was also evaluated in darkness. Under this condition the luminescence peak was shifted to about 4 times higher concentrations of Co^{2+} and Zn^{2+} in the *coaLux* bioreporter,

accompanied by a reduction of the maximal extent of luminescence value to approximately 65% and 50% of the respective values obtained after incubation in light. On the contrary, the *nrsLux* reporter strain did not show any luminescence when incubated in darkness with the tested concentrations of Ni^{2+}. The cause remained elusive.

The *ziaLux* bioreporter, previously generated in our laboratory, containing a full version of the repressor encoding-*ziaR* gene, was not functional in the tested conditions. We designed a second version of the *ziaR-O/*P_{ziaA}*-luxAB* fusions ($ziaLux_T$) that contained a truncated *ziaR* gene, lacking the last 308 base pairs. This construct showed also no luminescence activity upon incubation with Zn^{2+}. Aditionally, we created an As^{3+}/As^{5+}-responsive *arsLux* bioreporter. It responded to As^{3+} and As^{5+} in very different concentration ranges. The bioluminescent response was linearly dependent of the amount of As^{3+} from 8 µM to 500 µM. In the case of As^{5+}, the bioluminescent signal increased slowly from 2.3 mM to 10 mM As^{5+} and then at a higher rate up to 150 mM As^{5+} (the highest concentration tested). It is known that arsenic is a frequent contaminant of the groundwater used as a source of drinking water, sometimes at concentrations that greatly exceed the WHO drinking water quality limit for arsenic ions of 10 µg/l (0.13 µM). An example is the water from about 11 Hungarian settlements with a total population of about 24000, that contains arsenic levels higher than 50 µg/l (0.65 µM) [Jones et al. (2008) *Rev Environ Contam Toxicol* 197: 163], with a peak value of 560 µg/l (7.3 µM) [Csalagovics (1999) *Annu Rep Geol Inst Hung II*: 85]. In order to be applied for arsenic detection in such waters, the *arsLux* bioreporter needs further optimization for improving its sensitivity.

III. We have shown that the *artT* gene that encodes a putative chromate transporter from the CHR superfamily is cotranscribed with the neigbouring *slr5037* gene (designated by us as *artC*), which encodes a conserved, hypothetical protein. Upstream of the *artCT* operon and transcribed in the opposite direction, there are *sll5036* and *sll5035* genes (designated by us as *artS* and *artR*,

respectively), encoding a putative sulfide-quinone reductase and a putative SmtB/ArsR family metalloregulatory transcriptional repressor, respectively. Due to the involvement of this class of transcriptional repressors in metal ion sensing, the proximity of *ArtR* to the *artCT* operon and their opposite orientation, we have hypothesized that the *artR* gene encodes a repressor that regulates the expression of the *artCT* operon. Disruption mutants were generated in our laboratory by cloning a kanamycin resistance marker into *artR* and *artT* genes. The *artR*::Kan strain showed a constitutive derepression of the O/P_{artCT}, partially confirming our hypothesis. We found that the *artCT* operon was induced by chromate exposure when the sulfate content of the medium was low, as well as by As^{3+}. The *artT*::Kan strain was more resistant to As^{3+} than the wild type, as well as to chromate when grown in BG-11 that contains low levels of sulfate (10 µM). The *artR*::Kan disruption mutant was more sensitive than the wild type when exposed to As^{3+}. Taken together, these lines of evidence suggest that the protein encoded by *artT* works as a chromate/sulfate antiporter. At low sulfate concentrations the antiporter works as a chromate uptake system, therefore explaining the chromate resistance phenotype of the *artT* disrupted mutant. Weather ArtT is also able to transport As^{3+} remains an open question. The genomic context suggests that CHR proteins possess other physiological functions in addition to chromate transport (2007) [Díaz-Pérez et al. (2007) *FEBS J* 274: 6215].

IV. We established the concentration ranges for Cd^{2+}, Ni^{2+}, As^{3+}, Zn^{2+}, Co^{2+}, and Cu^{2+} in which *Synechocystis* cells respond specifically to the ion excess, as well as the concentration ranges in which general and oxidative stress responses occur. We investigated the transcriptional changes of specific inducible genes for each of these ions (except for Cu^{2+}, for which no specifically induced gene is known), and the following H_2O_2 or general stress-inducible genes, from the literature (as markers of general and oxidative stress): *isiA* (iron stress chlorophyll binding protein), *perR* (transcription regulator Fur family), *sigD* (group2 RNA polymerase sigma factor), *hspA* (16.6-kDa small heat shock protein), *dnaJ* (DnaJ-

like protein), *ahpC* (AhpC-peroxiredoxin), *lilA* (light harvesting-like protein), and *nblA1* (phycobilisome degradation protein). The results are summarized below:

Metal ion	Specific response	General and oxidative stress response
Cd^{2+}	2 µM	8 µM
Ni^{2+}	5 µM	10 µM
As^{3+}	80–720 µM	2 mM
Zn^{2+}	4 µM	16 µM
Co^{2+}	1 µM	–
Cu^{2+}	–	1.25 µM

No substantial stress response has occurred until the highest tested concentration of 32 µM Co^{2+}. The small heat shock protein-encoding hspA was highly induced by all tested metal ions at concentrations close to IC_{max}. The gene that encodes the chlorophyll-binding protein IsiA, previously shown to be activated by iron deficiency [Singh et al. (2003) *Plant Physiol* 132: 1825], was also induced by Ni^{2+}.

LIST OF PUBLICATIONS

Peca L, Kós PB, Vass I (2007) Characterization of the activity of heavy metal-responsive promoters in the cyanobacterium *Synechocystis* PCC 6803. *Acta Biol Hung* 58: 11-22.
IF: 0.688

Peca L, Kós PB, Máté Z, Farsang A, Vass I. (2008) Construction of bioluminescent cyanobacterial reporter strains for detection of nickel, cobalt and zinc. *FEMS Microbiol Lett* 289: 258-264.
IF: 2.021

(these publications are directly related to the subject of the thesis)

POSTER PRESENTATIONS

Peca L, Kós PB, Vass I (2006) Regulation of zinc, cobalt and chromium responsive genes in the cyanobacterium *Synechocystis* PCC 6803. 12th International Symposium on Phototrophic Prokaryotes. Pau, France, August 27-September 1st

Peca L, Kós BP, Vass I (2008) Development and utilisation of two bioluminescent reporter strains of *Synechocystis* PCC 6803 for detection of Ni^{2+}, Co^{2+} and Zn^{2+} contaminants. ESF-EMBO Symposium - Molecular Bioenergetics of Cyanobacteria: Towards systems biology level of understanding, San Feliu de Guixols, Spain, March 29-April 3rd

ORAL PRESENTATIONS

Peca L (2005) Quantitative analysis of *Synechocystis* sp. PCC 6803 gene expression in heavy metal stress. Genomics and Bioinformatics: Exploiting Microarrays in Plant Physiology, European Networking Summer School, Ljubljana, Slovenia, 22-31 August

Peca L (2005) Quantitative analysis of *Synechocystis* sp. PCC 6803 gene expression in heavy metal stress. EMBO Practical Course on Analysis and Informatics of Microarray Data, Cambridge, UK, 3-9 April