# Use of Macrolampis sp2 firefly Luciferase to detect heavy metal toxicity

Mariele Carvalho* and Vadim Viviani†[1,2]

[1]Graduate School of Biotechnology and Environmental Monitoring (UFSCar), Sorocaba, SP, Brazil (UFSCar) – Brazil

[2]Graduate School of Evolutive Genetics and Molecular Biology (UFSCar), São Carlos, SP, Brazil (UFSCar) – Brazil

**Abstract**

Heavy metals such as Pb, Hg and Cd cause severe impacts on the environment, however their biodisponibility is not easy assessed. Previously we showed that firefly luciferase can be used as a *light off* biosensor for heavy metals, and recently we showed that they can be harnessed to ratiometrically detect presence of heavy metals upon changes of intensities in the green and red regions of the spectrum. Therefore, we investigated the *in vivo* effects of heavy metals using recombinant bioluminescent bacteria carrying the luciferase gene of *Macrolampis* luciferase. We immobilized the transformed *E. coli* expressing *Macrolampis* sp2. luciferase in an agarose matrix in an ELISA plate. The heavy metals (ZnSO4, LiSO4, NiSO4, CdSO4 , PbCl2 and HgCl2) were added to the plate wells at the final concentration 1 mM and the plate was incubated for 12 hours at room temperature. The bioluminescence was imaged using a CCD camera and the bioluminescence spectra recorded using an ATTO spectrofluorimeter. The results showed that at the concentration of 1 mM, PbCl2 decreased the luminescente activity to _˜15%, whereas ZnSO4, LiSO4, NiSO4 inhibited the light emission up to 60%, 55% and 63% respectively. CdSO4 and HgCl2 displayed the largest effects, completely inhibiting the cell luminescence at 1mM concentration. A dose/effect curve showed that HgCl2, displayed a P50 of 25 $\mu$M, and all concentrations between 0,05 and 1 mM completely inhibited luminescence activity, whereas CdSO4 showed a P50 of 100 $\mu$M. The tetrazolium cell viability assay (TTC), confirmed the death of bacterial cells when exposed to heavy metals, correlating well with the luminescence results. Although, under these conditions we could not observe considerable differences in the *in vivo* bioluminescence spectrum of the cells we are investigating the effect of metals on bioluminescence spectrum at different exposure times.

**Keywords:** Luminescence, Heavy metals, Luciferase

*Speaker

†Corresponding author: viviani@ufscar.br