Suitability of *Macrolampis* Firefly Luciferase for *light off* Toxicity and Intracellular pH Biosensors

Gabriel, G.V.M.; Viviani, V.R.

Laboratório de Bioquímica e Biotecnologia de Sistemas Bioluminescentes, Pós-Graduação em Biotecnologia e Monitoramento Ambiental, Dep. de Física, Química, e Matemática, UFSCar, Sorocaba-SP, Brazil

**Introduction:** Fast and inexpensive monitoring techniques are increasingly required to assess water quality. Firefly bioluminescence is an ideal molecular life indicator to be used in biosensors, because it depends on ATP to produce light. The Brazilian *Macrolampis* firefly luciferase is classified in pH-sensitive, because its bioluminescence spectrum shifts from yellow-green (564 nm) to red (610 nm) at acid pH. With the aim to develop *light off* cell biosensor to analyze general toxicity, we have prepared bioluminescent *E. coli* transformed with plasmids carrying cloned luciferase cDNA of *Macrolampis* and tested the effect of heavy metals, desinfectants and different pHs.

**Material and Methods:** We realized *in vitro* and *in vivo* luminometric and spectrofluorimetric analysis with *Macrolampis* firefly luciferase in phosphate buffer pH 6 and 8, with heavy metals and desinfectants, and analyzed the ratio of the intensity 610 and 550 nm.

**Results and Discussion:** Despite a higher sensitivity to Hg^{2+} and Cu^{2+}, the results show that in general the wild-type *Macrolampis* firefly luciferase do not suit for specific heavy metals detection, although it can be used for general toxicity *light off* biosensor with sensitivities similar to Biolux kit employing naturally bioluminescent *Vibrio fischeri* bacteria, especially to cresol red, iodine, 1-propanol and potassium dichromate. A relationship between pH and the ratio of intensity of bioluminescence spectra at 550/610 nm was found, showing promising applicability of this luciferase for ratiometric intracellular pH-sensing.

**Conclusion:** While bioluminescent bacteria transformed with firefly luciferase do not show specificity for any class of toxicants, it may be potentially useful as general toxicity *light off* biosensors. On the other hand, the relationship between pH and bioluminescence spectra intensities at different wavelengths shows potential applicability of this luciferase for simultaneous intracellular pH-sensing and ATP estimation.

Key words: luciferase, biosensor, bioluminescence

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