Expression and characterization of mutants affecting with pH-sensitivity of *Macrolampis* SP firefly luciferase.

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**Introduction:** Luciferases are enzymes that lead to production of bioluminescence in different organisms, catalyzing the oxidation of molecules generally known by luciferins producing a photon of visible light with high efficiency. In pH-sensitive firefly luciferases, several mutations in their primary structures are known to affect the emission spectra, in general shifting to the red. Aiming to understand which regions of *Macrolampis* sp2 luciferase are related to pH-sensitivity, site-directed mutagenesis have been carried out.

**Material and methods:** Site-directed mutagenesis was performed using a Stratagene mutagenesis kit, the plasmid containing the luciferase cDNA was amplified using Pfu polymerase and complementary primers containing the desired mutation. Bacteria were transformed with the mutant were grown in liquid cultures (O.D. 600 = 0.4) and induced with IPTG. The recombinant luciferase was extracted and its *in vitro* bioluminescence activity and spectra were luminometrically and spectrofluorimetrically assayed in presence of D-luciferin, Mg-ATP and buffer. Kinetic characterization was accomplished using different concentrations of luciferin and constant concentration of Mg-ATP, after that was used different concentrations of ATP and constant concentration of luciferin. **Results and discussion:** The mutant Mac S314C (λ max=578[100]nm) had its spectrum broadened and slightly red-shifted in relation to the wild-type luciferase (λ max=560[66]nm), whereas the mutant Mac F250S (λ max=562[76]nm) had its spectrum narrowed and shifted to the red. The values of KM underwent significant changes: the KM for luciferin decreased for S314C mutant (wild-type=19µM; S314C=7µM) and the KM for ATP decreased for both mutants (wild-type=83µM; S314C=18µM; F250S=12µM). **Conclusion:** These mutagenesis studies, as well as site-directed mutagenesis studies with other beetle luciferases and modelling studies indicate that the residue S314 might be involved in a main-chain interaction with oxyluciferin phenolate, modulating bioluminescence colors, whereas S250 in green emitting luciferases apparently stabilizes the active site.

**Keywords:** Luciferase, *Macrolampis*, pH-sensitivity

Financial Support: CNPq