THERMOSTABILITY OF BRAZILIAN BEETLE LUCIFERASES: SUITABILITY OF Pyrearinus termitilluminans LUCIFERASE FOR in vivo CELL ASSAYS

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Beetle luciferases catalyze the ATP activated oxidation of firefly luciferin, producing oxyluciferin and bioluminescence with high efficiency. They are very important bioanalytical reagents for ATP measurements. However, their commercial use as bioanalytical reagents and as reporter genes is limited by their intrinsic low stability. Currently commercially available reagents and kits use a stabilized version of the North-American *Photinus pyralis* firefly luciferase. Therefore, the selection of stable luciferases with different properties and their stabilization under different circumstances is desirable. Using beetle luciferases previously cloned in our laboratories, we investigated their thermostability and kinetics. Among them, the luciferases from *Pyrearinus termitilluminans* and *Macrolampis* sp2 were the most stable, and therefore were selected for further studies. *Pyrearinus termitilluminans* luciferase displayed a slow luminescence kinetics (K_D= 4,5 . 10^{-3} s^{-1}) and was the most stable, with half-life of 5 days at 4°C, 2 days at room temperature and 5 h at 37°C. Frozen enzyme kept at -20°C retained full activity for over 10 months. Furthermore, *in vivo* studies with *E. coli* cells expressing these recombinant luciferases, showed that *Pyrearinus termitilluminans* luciferase displays a sustained luminescence, which is especially stable to thermal inactivation when incubated at 50°C, whereas *Macrolampis* luciferase displays a flash like kinetics which is less stable and spectrally sensitive to temperature. These results indicate that *Pyrearinus termitilluminans* is appropriate for *in vivo* reporter and cell bioimaging studies. (Financial Support: CNPq and FAPESP) key-words: luciferases, Pyrearinus termitilluminans, thermostability.