Developing a beetle luciferase-based bacterial bioluminescent biosensor for water toxicity analysis

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Fast and inexpensive monitoring techniques are increasingly needed to assess water quality. Beetles luciferases are ideal molecular indicators of life, because they depend on ATP to produce light. In order to develop light off cell biosensor to analyze water toxicity, we are constructed a bioluminescent E. coli transformed with plasmids carrying two cloned beetle luciferases: Pyrearinus termitilluminans which emits green light (538 nm) and produce an intense and continuous luminescence and Macrolampis which produce a pH and cation-sensitive bioluminescence, with fast decaying luminescence, whose spectrum shifts from orange (600 nm) to yellow (569 nm). The luminometric assay detected up to 10 cfu/mL whereas the CCD camera 100 cfu/mL, showing that this method is quite sensitive. The bioluminescence signal of transformed E. coli is comparable or superior than Biolux kit (naturally bioluminescent bacteria). We tested herbicide and heavy metals, CuSO₄ and ZnSO₄ displaying the strongest inhibitory effects on bioluminescence in vivo assay and bacterial growth in agar diffusion test. However, beetle luciferases also show luminescence inhibition with different ZnSO₄ concentrations, indicating that our biosensor is not very useful for specifically detecting heavy metals in its current format. Finally, in order to have a more stable assay for field measurements, we have immobilized the bioluminescent bacteria in agarose in an Elisa plate. In this format, immobilized bacteria keep strong bioluminescence activity for a 3 weeks when stored at 4° C. Altogether, these results show the potential beetle luciferase-based E. coli bioluminescent biosensor for fast water toxicity analysis, although further optimization is needed.

Keys Words: luciferases, biosensors, bioluminescence

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