Quantification of Toxicity Using Bioluminescent Bacteria and an Instant Film Camera

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OBJECTIVE

- Development of an easy to use, rapid, cost-effective, and portable bioluminescence toxicity detection system using Vibrio fischeri strain NRRL B-11177.

INTRODUCTION

- Assays based on the measurement of bacterial luminescence are widely used in ecotoxicology. The most common is the measurement of inhibition of bioluminescence from the bacteria Vibrio fischeri strain NRRL B-11177.
- Bioluminescence reflects the metabolic status of the bacteria and will decrease when the bacteria cells are exposed to toxic substances.

METHODS

- Materials
  - Chemical standards were obtained from Sigma (St. Louis, MO) or Acutest (New Haven, CT). Vibrio fischeri/strain NRRL B-11177.
  - Equipment
    - Camera Exposure Chamber, Luminometer, Incubation Chamber, Abraxis (Warminster, PA).
    - Polaroid film 667, ISO 3000/DIN 36.
    - Scanner, Epson 3590 (Japan).
    - ImageJ quantification program, National Institute of Health (Bethesda, MD).
    - Motorola V551 Cell Phone.

RESULTS AND DISCUSSION

- Proper Exposure time for the film was chosen to be 2-5 minutes (Fig. 3).
- < 2.5% was the concentration of Methanol chosen for the dilution of less soluble compounds (Fig. 4).
- Some differences in toxicity were found between the camera and luminometer when metals were tested. Subsequent experiments indicate incubation temperature of bacteria has a pronounced effect on the EC50 of metals with room temperature incubation exhibiting a greater inhibition of luminescence than incubation at 15 ºC (Fig. 5).

CONCLUSIONS

- This work describes the quantification of luminescence from luminescent bacteria (Vibrio fischeri/strain NRRL B-11177) by the use of a Polaroid film. The use of film for quantification offers significant advantages over the use of a luminometer: it’s inexpensive, easy to use, and requires no external power. The film can be inspected visually immediately and it can also be stored as a hard copy of the results.
- Computer aided quantification from Polaroid film can yield comparable results to luminometer values as long as incubation temperatures of the bacteria are similar (15 ºC) for both systems.
- Results obtained using a cell phone with camera and computer aided quantification were different to values obtained by direct scan of the photo. Indications are that those discrepancies are due to photo glare and improper scanner settings. More work is being conducted in this area.
- We have shown that it is possible to detect luminescence from ecotoxological tests based on bioluminescent bacteria using inexpensive instrumentation. This type of toxicological test system is ideally suited for developing countries.