

August 1969

Brief 69-10294

NASA TECH BRIEF



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Mass Culture of Photobacteria to Obtain Luciferase



The problem:

Devise a laboratory method for mass culture of aerobic microorganisms, particularly photobacteria, to obtain large quantities of luciferase.

The solution:

Inoculate large preheated trays filled with nutrient agar with photobacteria and grow these microorganisms in a controlled environment. Monitor growth by automated light-detection instrumentation to deter-

mine optimum harvest time. Selectively scrape the luminescent organisms from the agar surface with a rubber spatula in the dark and process the cell paste obtained to prepare luciferase.

How it's done:

Five strains of photobacteria are used, namely, *Photobacterium fischeri*, *P. harveyi*, *P. pierantonii*, *P. phosphoreum*, and *Vibrio albensis*. Stocks are routinely cultured on a minimal medium.

(continued overleaf)

Warm sterilized fiberglass trays, 50 × 75 × 4.5 cm, are filled to a depth of one centimeter with three liters of hot sterile agar medium, covered loosely with aluminum foil, and allowed to cool on a level surface. One hundred milliliters of a dense, luminescing culture are poured on the cooled agar tray and spread thinly and evenly with a bent glass rod or cotton swab. The tray is placed into the facilities used for temperature and humidity control.

A polymethyl methacrylate plate bridges the agar surface to support a light sensor. Covering the entire tray with heavy-duty aluminum foil keeps the surface dark and minimizes evaporation and contamination during growth. A commercially available photomultiplier in a photometer monitors changes in luminescence. The photomultiplier output feeds into a logarithmic amplifier to record the wide range of light produced over maximum growth. This amplifier compresses seven decades of response into a 0 to 10-volt output signal recorded on a strip-chart recorder with a mechanical timer set for recording 5 seconds of each 15-minute period. This allows continuous monitoring over any desired time period. The photograph shows the entire apparatus.

Trays are harvested at maximum luminescence in the dark to facilitate selection of the most luminous areas for scraping with a rubber spatula. Three liters of medium can yield as much as 100 grams of Photobacterium-cell paste, which can be processed to prepare enough luciferase for a large number of experiments.

Notes:

1. Laboratories and small-scale pilot plants with limited facilities will find this method for mass culture of aerobic microorganisms very useful. Numerous biological components may be extracted from the microorganisms.
2. Additional information may be found in a paper, "A Simple Method for the Mass Culture of Microorganisms with Reference to Photobacteria", by G.L. Picciolo, E.W. Chappelle, and E. Rich, Jr., *Applied Microbiology* 16, 954-955, June 1968.
3. No further documentation is available.

Patent status:

No patent action is contemplated by NASA.

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