Simple Device for Enumeration and Isolation of Luminescent Bacterial Colonies

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By virtue of their ability to emit visible light, luminous bacteria have become not only biological curiosities, but have also provided much comparative data on basic biological processes. The detection and subsequent isolation of luminescent bacteria involves the examination of platings of natural material (e.g., seawater) or the surface of a solid substrate such as decomposing fish flesh. These materials must be observed in a dark enclosure before the developed colonies can be selected and transferred to a suitable medium. In platings containing many cells, the process of identifying and picking luminescent colonies is often difficult or, at least, inconvenient in a totally dark room. The device described permits a clear-cut distinction between luminescent and nonluminescent isolates, even on plates containing several hundred bacteria.

A Quebec bacterial colony counter (American Optical Co., Buffalo, N.Y.) was fitted with a 10-watt red light bulb, and connected to a laboratory powerstat (rheostat). The unit was used in a darkened area, i.e., darkroom, closet, or incubator room. The plate to be examined was placed on the counter, and the powerstat was turned to maximal illumination, allowing the plate and colonies to become easily discernible in the absence of external illumination. The intensity of red light was diminished by reducing the powerstat setting, and, at a greatly reduced degree of red illumination, colonies were easily distinguished; nonluminescent ones became only slightly visible, and those luminescing assumed a bluish tinge, accentuated by the red light. Sufficient illumination was present to allow a careful circling (or other designation) of luminous colonies by use of a marking pen or pencil. Under normal lighting, those designated could easily be picked for subculture.

In our experience, a medium composed of 0.5 % Gelysate (BBL), 0.01 % yeast extract, 0.01% FePO_4, and 1.8% agar in full strength seawater was suitable for the isolation of marine luminescent bacteria. Cultures have also been maintained on this medium in screw-cap tubes for up to 2 years without transfer at 20°C, and continue to luminesce on transfer to fresh medium. The possible presence of organic matter such as aldehyde (J. Spudick and J. W. Hastings, J. Biol. Chem. 238:3106, 1963) in the seawater diluent used warrants attention.

Luminescent bacteria have been used as indicator organisms in air pollution studies (W. F. Serat, F. E. Budinger, and P. K. Mueller, J. Bacteriol. 90:832, 1965), and their application to similar problems in aqueous environments seems logical. The device described provides a simplified technique for the rapid enumeration of luminescent bacteria as a portion of an indigenous or artificial flora.

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