[20] A "Bucket of Light" for Viewing Bacterial Colonies in Soft Agar

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Abstract

The morphologies of bacterial colonies in soft agar media can provide a wealth of information about a strain's locomotor and chemotactic abilities. Photographic images are often the simplest and most effective means of documenting these behavioral phenotypes. Uniform, indirect, transmitted illumination of the plates is essential for obtaining good colony images. This brief chapter describes a simple and relatively inexpensive illumination device for viewing and photographing bacterial colonies in soft agar.

Viewing Colonies Grown in Soft Agar

Soft agar assays for motility, chemotaxis, and aerotaxis are described in detail elsewhere in this volume (Ames and Parkinson, 2007; Taylor *et al.*, 2007). Briefly, in soft agar media, flagellated bacteria such as *E. coli* swim within the water-filled tunnels in the agar matrix. The expanding colony typically extends from the bottom of the plate to the top surface of the plate with the majority of the cells embedded in the agar rather than at the air interface. These colonies can contain one or more rings of cells that are concentrated near the outer boundaries of nutrient, energy, or electron-acceptor gradients generated through the cells' metabolic activities. The size, thickness, relative position, and depth of each ring provide important information about a strain's motility and tactic behavior.

Direct light from above is ill-suited for viewing or photographing soft agar colonies. Cells on the surface of the agar reflect much of the incident light, whereas those within the agar receive and reflect much less light. Consequently, this type of illumination obscures much of the detailed structure of the colonies (Fig. 1A). In contrast, indirect light from behind the agar more evenly illuminates the colonies and brings out their fine structure features, such as internal rings or fuzzy edges (Fig. 1B). However, the light should not pass from the source directly upward through the plate, but rather should enter only the bottom half of the plate and at an oblique angle, approximating a dark-field effect.

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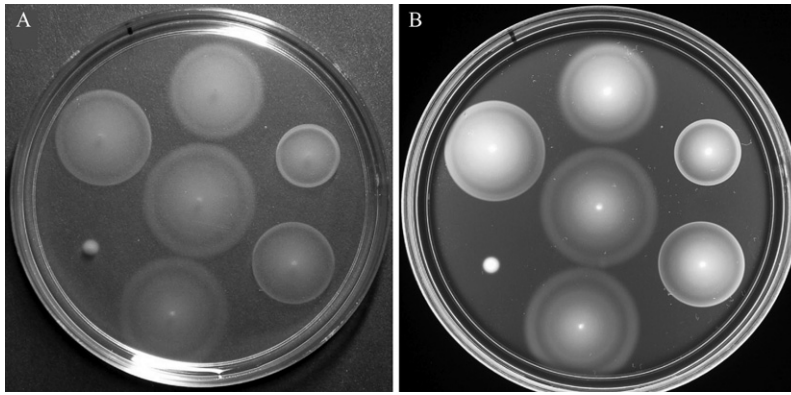

FIG. 1. Colonies in soft agar photographed with reflected (A) or transmitted (B) light. Isogenic *E. coli* strains with different chemotaxis phenotypes were inoculated into tryptone soft agar medium and incubated at 30° for 8 h. In panel (A), the plate was illuminated with incident fluorescent light from a desk lamp. In panel (B), the plate was illuminated with the bucket of light described in this chapter. Both photographs were taken with a digital camera at the same magnification and resolution and at an automatic exposure setting chosen by the camera.

Building a Bucket of Light

A dark-field illumination source for viewing bacterial colonies in soft agar plates can be built by a metalworking shop for less than $300. A design used by a number of research groups employs a circular fluorescent tube [22W Sylvania rapid-start cool white, model FC8T9 or equivalent] inside a cylindrical metal container (Fig. 2). Aluminum is the preferred material because of its relatively light weight, but other metals should also work. The cylinder is formed from a 1/8″ (~2 mm) plate, approximately 18 × 83 cm, with the rolled-up edges joined by brazing. Enterprising researchers might be able to find ready-made circular containers with roughly the same dimensions, for example, a metal wastebasket. In our experience, none of the dimensions is very critical for good performance.

Both ends of the cylinder are covered by circular plates made from 1/4″ (~6 mm) aluminum plate, but other materials could probably be substituted, particularly for the bottom plate. The top plate contains a circular hole ~82 mm in diameter that has a machined lip ~2 mm deep and 3 mm wide for supporting a standard-size plastic Petri dish (see enlarged cross-section view in Fig. 2). The dimensions of the viewing hole could presumably be changed to accommodate other sizes of culture plates, perhaps even square plates, although we have not tried them. The bottom plate is primarily intended to protect the electronics for the fluorescent tube and can

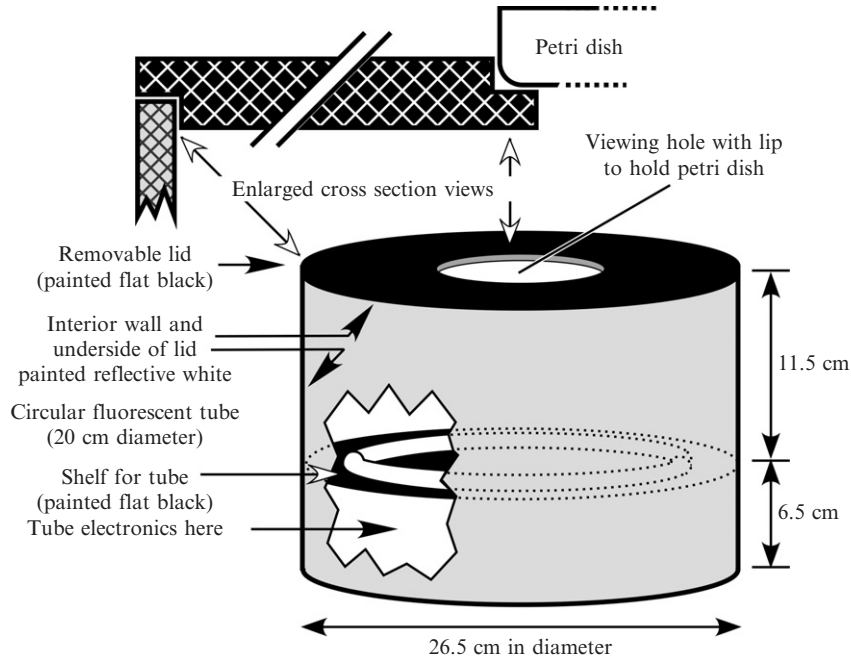

FIG. 2. Illuminator for viewing bacterial colonies in soft agar plates. The nature and dimensions of the stock material are provided in the text.

be mounted to the cylinder by brazing, machine screws, or some other means. The top plate should be removable for cleaning and maintenance access. This can be accomplished by machining a lip around its outside edge that allows it to nest on the top and against the inside edge of the cylinder wall (see enlarged cross-section view in Fig. 2).

The fluorescent tube is supported by clips mounted to a solid circular platform positioned approximately 1/3 of the overall height from the bottom of the cylinder. This platform can be mounted with machine screws (for maintenance access) to a few support posts brazed to the inside wall of the cylinder. The electronics for the fluorescent light are installed below this platform, which can be made from aluminum or any other suitable material. The upper surface of the platform is spray painted flat black to provide a dark, nonreflective viewing background. The inside wall of the cylinder and the underside of the lid should be spray painted with flat white paint to diffuse the light and to provide uniform intensity within the field of view.

In use, the fluorescent tube should not be visible when looking straight down through the viewing hole. If this proves to be a problem, a cylindrical

shield (13–15 cm in diameter and 3–6 cm in height) can be made from black cardboard and placed on the tube support platform.

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References

Ames, P., and Parkinson, J. S. (2007). Phenotypic suppression methods for analyzing intra- and inter-molecular signaling interactions of chemoreceptors. *Methods Enzymol.* **423,** 436–457.

Taylor, B. L., Watts, K. J., and Johnson, M. S. (2007). Oxygen and redox sensing by two-component systems that regulate behavioral responses: Behavioral assays and structural studies of Aer using *in vivo* disulfide crosslinking. *Methods Enzymol.* **422,** 190–232.