Alternative Procedure to Screening a Large Number of Plates for a Low Number of Mutants

DENNIS C. WESTHOFF

Department of Dairy Science and Food Science Curriculum, University of Maryland, College Park, Maryland 20742

Received for publication 21 September 1973

An apparatus is described in which tygon tubing replaces the use of petri dishes in the screening of a large volume of agar for an expected low number of mutants.

By employing conditions for optimal mutation of Streptococcus lactis as previously described (2), we were attempting to isolate mutants which would form colonies on lactic agar (1) within 48 h at 32 C when the medium was adjusted to pH 5.1. Tempered lactic agar was inoculated with the mutant population and then poured into a large number (200 to 300) of petri dishes and incubated at 32 C. After 24 h of incubation, the plates were removed and observed for colony formation at 6-h intervals up to 48 h. This required removing several hundred plates from the incubator, placing them on a Quebec colony counter, observing for any colony formation, and then replacing the plates in the incubator. In an attempt to eliminate this time-consuming task, we have designed the apparatus shown in Fig. 1. It functions much like a film editor in that by turning the two plexiglass wheels, tubing containing inoculated lactic agar can be viewed either by a low-power dissecting microscope or with the naked eye and colonies detected. The desired colonies can easily be isolated by scrubbing that area of the tubing with a quatincture (0.1% hyamine in 70% ethanol) and then piercing the wall with a sterile 18-gauge hypodermic needle and fishing the colony out. The section of the tubing pierced cannot be reused. We usually just removed that section and rejoined the tubing with a connector. A smaller gauge needle might not damage tubing, but by using an 18-gauge needle, the colony is retained somewhat inside the needle. To eliminate any other possible contamination removed with the needle, the culture can then be streaked onto the original isolation medium for confirmation.

The actual dimensions are not critical. The apparatus depicted in Fig. 1 is made entirely of 0.5-inch (1.27 cm) plexiglass. The two drums for winding the tubing are 30.5 cm in diameter, with the space between the wheels being 15 cm. The complete base plate is 30.5 by 61 cm.

A more detailed description of the operation is as follows. Two 50-ft (15.24 m) sections of 3/4-inch (0.794-cm) outer diameter tygon flexible tubing (a plasticized polyvinyl chloride tubing) were joined together with a QD connector (Malinckrodt, New York). The two open ends of the resulting 100 ft (30.48 m) of tubing were cemented with epoxy glue to predrilled holes in each of the wheels via a QD connector. The tubing was sterilized by attaching another piece of similar tubing with connector (labeled as B in Fig. 1) to a reservoir of quatincture and drawing approximately 2 liters of quatincture through the tubing by attaching flask A to an aspirator. The quatincture was followed by 4 liters of sterile distilled water at 90 to 95 C. Lactic agar (pH 5.1) was tempered to 43 C, inoculated with the mutant population, and

Fig. 1. Apparatus used to wind tygon tubing containing inoculated agar. A, Filtering flask attached to aspirator; B, tubing connection for sterilizing and filling with agar.
immediately drawn into the tubing in a similar fashion. The agar was permitted to solidify within the tubing before the apparatus was placed in a 32-C incubator. Flexibility of the tubing is maintained when the agar solidifies. The two pieces of tubing needed for sterilizing and filling the tubing with agar, that is, the tubing labeled B in Fig. 1 and the tubing from the small vacuum flask labeled A in Fig. 1, were removed before incubation, and the connector was simply covered with tape. After the desired intervals of incubation, the apparatus was removed from the incubator and the wheels were turned to permit viewing of the entire length of tubing. In most cases, colonies were detected with the naked eye; however, to confirm the presence of a colony and for isolation, the low-power dissecting microscope was used, as shown in Fig. 1. The agar can be removed by slowly pressurizing tubing B (Fig. 1) and pushing the agar out into a container placed at outlet A. The tubing can be reused repeatedly.

The apparatus as described has proven to be quite successful for our purposes, and we have isolated mutants. By employing longer lengths of tubing (these wheels will hold about 300 ft (91.44 m) of \( \frac{3}{8} \) O.D. tubing), we can incorporate about 2.5 liters of lactic agar, which would be equivalent to 200 petri dishes. In addition, the time involved in handling plates has been eliminated. The apparatus has also made it practical to remove the inoculated agar at more frequent time intervals from the incubator in order to detect the first visible colony.

Any technique employing a large number of plates in the screening of an expected low number of mutants would probably benefit from the use of this apparatus. Problems may be encountered with highly motile organisms (spreading in tube) or with strict anaerobes (O\(_2\) permeability of the tubing). We have attempted neither, but for the latter (anaerobes), tubing other than tygon, or possibly the addition of a reducing agent to the medium, might be more suitable. We have used the apparatus for other purposes; for example, by inoculating the agar with more than one culture we have been able to observe inhibitory reactions between cultures by looking for zones of inhibition within the tubing. In this way, large populations can be screened.

**LITERATURE CITED**

1. Eliker, P. R., A. W. Anderson, and G. Hannesson. 1966. An agar culture medium for lactic acid streptococci and lactobacilli. J. Dairy Sci. 39:1611-1612.

2. Westhoff, D. C., and P. Adams. 1972. Survival of *Streptococcus lactis* and *Leuconostoc citrovorum* following exposure to N-methyl-N-nitro-N-nitrosoguanidine. J. Dairy Sci. 55:865-866.