NEW USES FOR MEMBRANE FILTERS

II. ISOLATION OF AUXOTROPHIC MUTANTS

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The advantages of using solid media throughout the penicillin procedure for the selection of many auxotrophic (i.e., nutritionally deficient) bacterial mutants of discrete mutational origin were pointed out by Adelberg and Myers (J. Bacteriol. **65**:348, 1953). Their procedure requires six layers of agar, the isolation of colonies inbedded under four layers of agar, and several extensive periods of refrigeration to permit diffusion of penicillin, penicillinase, and nutrient supplements. The present report describes a simplified procedure which achieves the same objectives by using membrane filters. Preliminary studies were done several years ago with *Escherichia coli* B/r (Matney and co-workers, unpublished data); a stable LT-7 strain of *Salmonella typhimurium* was employed in the present investigation.

About 2×10^7 log-phase cells grown in nutrient broth were impinged on each of several membrane filters (Matney, Shankel, and Wyss, J. Bacteriol. **75**:180, 1958). The membranes were placed on the surface of minimal agar (Vogel and Bonner, J. Biol. Chem. **218**:97, 1956), exposed to sufficient ultraviolet light to reduce the number of viable cells per membrane to $<10^5$, and incubated at 37 C for 6 hr. The membranes were transferred to minimal agar plates containing 200 units of penicillin per ml and reincubated at 37 C overnight. After placing the membranes on

fresh minimal agar for 1 hr to permit the residual penicillin to diffuse away from the membranes, they were transferred finally to minimal agar supplemented with 0.02% dehydrated nutrient broth. After 1 to 2 days of incubation, the small colonies which developed were picked and tested for their nutritional requirements.

The ultraviolet exposure and the incubation time on minimal agar prior to penicillin treatment are two variables which may have to be varied with different bacterial strains to achieve maximal mutant yields.

Experiments were performed with a mixture of histidineless auxotrophs and prototrophs to determine the amount of nutrient broth supplementation that permitted the auxotrophic cells to form small colonies on membrane filters. Similar experiments with limiting concentrations of L-histidine suggested that enrichment with $\frac{1}{40}$ the concentration required for maximal growth will reduce the colony size of an auxotroph so that it may be distinguished from the wild type. Specific supplementation rather than nutrient broth permitted the direct isolation of several auxotrophic types.

The membrane filter technique has been used to isolate mutants of *Salmonella* with growth requirements for leucine, histidine, cysteine, methionine, tryptophan, tyrosine, serine or glycine, and thiamine. The variety of mutants isolated suggests that there is little or no selection for a limited class of auxotrophs with this procedure.

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III. BACTERIAL MATING PROCEDURE

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A procedure has been developed in which *Escherichia coli* K-12 donor (*HFr*) and recipient

(*F*⁻) bacteria are permitted to conjugate on the surface of membrane filters. The rapid separation

of conjugal pairs encountered in broth matings is virtually eliminated, since the cells are positionally fixed during the mating process.

Difco Nutrient Broth cultures of the parental strains were grown overnight at 37 C with aeration, diluted 1:20 in fresh broth, and the incubation continued for 2 hr. The culture flasks were placed in an ice bath to arrest growth. The cells from 4.5 ml of the F^- culture and 0.5 ml of the donor culture were impinged (Matney et al., *J. Bacteriol.* **75**:180, 1958) onto each of several membrane filters (about 10^8 donor cells and 10^9 recipient cells per membrane). The cells were washed twice by reflooding the membrane with small portions of saline (0.8% NaCl). The membrane was removed from the filter device and stored on the surface of cold minimal agar plates (Vogel and Bonner, *J. Biol. Chem.* **218**:97, 1956) until the desired number of membranes were prepared. The mating processes were initiated by rapidly transferring the membranes from the cold plates to the surface of prewarmed (37 C)

soft (0.75% agar) minimal plates supplemented with 10 μ g of vitamin B_1 /ml. Chromosomal transfer could be arrested at any time by removing a membrane from a mating plate and placing it in a flask containing 10 ml of cold saline. The cells were suspended by shaking the flask. Conjugal interruption was effected by the usual blending or virulent-phage treatments (Adelberg and Burns, *J. Bacteriol.* **79**:321, 1960). Suitable dilutions of the mated cell suspension (usually 10^{-5} with respect to the original male culture) were plated on selective agar media and the recombinant clones scored after 2 days of incubation at 37 C.

This procedure permits the complete donor genome to be transferred with high frequency. (About one-third as many terminal recombinants are recovered as for lead markers.) Entrance times may be determined for any marker on the chromosome of either stable *Hfr* or *F'* donor types.

SYNTHETIC FIBER BATTINGS AS PLUGGING MATERIALS IN MICROBIOLOGY

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Three synthetic fibers are now being used as substitutes for cotton as filling material in furniture, and are available as battings. These are Dacron polyester fiber, Orlon acrylic fiber, and acetate. Of these four fibers (the three synthetics and cotton), Dacron fiberfill has the lowest moisture content and moisture regain throughout the entire humidity range (Technical Information Bulletin D-112, 1960, E. I. DuPont de Nemours & Co., Inc., Wilmington, Del.). According to this bulletin, Dacron fiberfill is odorless and nonallergenic, and is not weakened by microorganisms or attacked by insects. It does not flash burn, but melts as it burns when a flame is applied. The burning "melt" usually falls away and extinguishes itself.

The current price of Dacron fiberfill batting is \$1.32/lb, but it is lighter than plugging cotton batting. Some advantages of Dacron batting as a plugging material in microbiological work are that the fibers are stronger and more adherent than those of plugging cotton, and thus the Da-

cron plugs hold their shape better and are more often reusable than cotton after autoclaving or heat sterilization. The Dacron plugs loosen slightly while becoming shaped during the first autoclaving; thus, a tight plug can be made originally. The plugs are wettable if water is poured on them, but they do not readily absorb water during autoclaving of liquid media. The end of the plug in the flask becomes slightly damp, but this moisture is later released. When immersed in water and then wrung out, the Dacron fiberfill readily releases the water and does not form a wad (as does absorbent cotton). The main advantage of Dacron as a plugging material is its heat resistance. Dacron plugs can be sterilized for 2 hr with dry heat (180 C) at least four times without significant discoloration.

The cheaper (\$0.70/lb) but heavier acetate batting also forms plugs which hold their shape better than those of cotton, and release the slight amount of moisture dampening the end of the plug during autoclaving of liquid media. How-