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NOTES

Suggested Procedure Allowing Use of Plastic Petri Dishes in Bacteriocin Typing

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After bacteria are mechanically removed from solid media, the remaining viable cells can be killed by exposure to chloroform vapors. Until recently, the applicability of this procedure was restricted to glass petri dishes. Here a procedure is described in which plastic petri dishes are used and remain stable in the presence of chloroform vapors.

Typing of bacterial isolates according to their production of bacteriocins has become an important tool in epidemiological research, especially for hospital-acquired infections with *Pseudomonas aeruginosa*.

In general, the method follows the principle developed by Gillies and Govan (3) and modified by Bergan (2) and Bauernfeind et al. (1). Briefly, the strain tested for pyocine production is grown under appropriate conditions on solid medium. During growth, pyocines are set free from the cells and pass into the agar. After 14 to 18 h of incubation, the producer cells are scraped from the surface of the medium, and any remaining cells are killed by exposure to chloroform vapor. Since plastic petri dishes soften and lose their transparency in a chloroform atmosphere, glass petri dishes have been necessary for this procedure. This, of course, incorporates the known disadvantages of glass petri dishes as compared with plastic materials (e.g., higher cost and weight, unevenness, and necessity of cleaning and sterilization). We thus developed a procedure allowing the use of plastic petri dishes.

Viable producer cells left behind on the agar surface are killed in the following way. A circular piece of filter paper (e.g., Schleicher and Schüll no. 595, ϕ 7 cm) is laid in the middle of a glass petri dish and saturated with 1 ml of chloroform. None of the $CHCl_3$ should run off the filter paper onto the glass. The agar plate is then inverted and laid over the filter paper (Fig. 1), so that the free agar surface is exposed to the $CHCl_3$ vapor, and left there for 15 min at room temperature. This is sufficient to kill all remaining bacteria. After 15 min the petri dishes, still inverted (but left open) over their own plastic lids, are placed

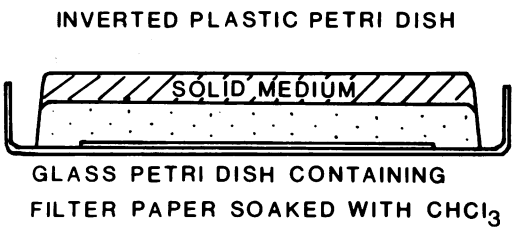

FIG. 1. *Device for killing bacteria in plastic petri dishes by exposure to chloroform vapors.*

for an additional 15 min in a 37°C incubator. This ensures complete removal of any remaining $CHCl_3$ vapor. The plates are then prepared for streaking with the indicator strain.

In this procedure, the greater part of the plastic petri dish does not come into contact with the chloroform vapor; only the upper half of the inner side wall is exposed. According to our experience with more than 10,000 plates, the shape of the dish is not distorted. The bottom of the dish is completely protected from the influence of $CHCl_3$ by the medium and thus retains its stability and transparency. This procedure may make the typing of *P. aeruginosa* by pyocine production more practicable.

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