

## Apparatus for Rapid Replica Plating in Rhizosphere Studies

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Rhizosphere investigations are restricted by the sheer number of cultures that must be handled in the multitude of different tubed media needed to characterize each isolate. A current rhizosphere study called for the use of 25 different diagnostic media with each of hundreds of isolates, a burdensome and laborious problem. Moreover, the replica plating technique of J. Lederberg and E. M. Lederberg (*J. Bacteriol.* **63**:399, 1952) was unsatisfactory, because colonies were smeared when transferred by use of a velveteen pad after five or six plates were replicated from the master. Replication of 25 plates, each containing differential media, therefore required five masters. Because a more satisfactory method was needed, a replicator was devised adapting certain features of the replicating apparatus of G. Stotzky (*Can. J. Microbiol.* **11**:629, 1965) and of C. Quadling and R. R. Colwell (*Can. J. Microbiol.* **10**:87, 1964). A grid of inoculating needles was attached to a press (Fig. 1) to serve as a replicating inoculator.

The replicator consists of three main parts. The press is a bottle capper, modified to fit into a wooden frame (Fig. 1A). The replicating part (Fig. 1B) is cut to the size of a petri dish from 16-gauge galvanized sheet metal. Brass nails (1.3 cm long) were inserted through and soldered in small holes drilled through the metal, 1.3 cm apart. The third part (Fig. 1C) consists of a sliding platform supporting two wood stands. One functions as a culture tube rack, the tubes being spaced 1.3 cm apart to match position of the brass nails. The other stand holds the petri dish into which the nails carrying inocula are to be imprinted. To prevent contamination, a sterile petri-dish lid covers the array of culture tubes when not in replicating position. In operation, 1-ml culture tubes containing suspensions of isolates obtained by standard methods are slid to the right under the metal replicator which is then depressed, each brass nail serving as an inoculation needle. The culture tubes are then covered with the protective petri lid, and the stand holding the dish to be inoculated is then slid to the left under the replicator. Depression

of the replicator imprints the agar medium with an inoculum of each culture. Replication of 25 cultures occurs simultaneously; the process may be repeated as often as desired.

The metal replicator may be autoclaved or flamed in alcohol when cultures are changed.

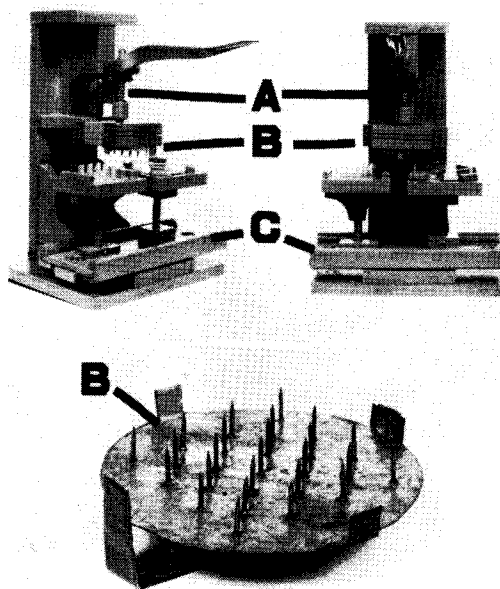


FIG. 1. Replicating inoculator. (A) Press, with block to hold inoculating needle; (B) inoculating needles soldered in metal disc; (C) sliding platform supporting rack of culture tubes and petri dish to be inoculated.

Flat-bottomed tubes (8 by 30 mm) were best suited for the replicator. Contamination, readily detected on inoculated plates by position out of the nail pattern, is minimal, especially when plates are replicated in a culture hood. For replication of 1,000 rhizosphere isolates on 10 different diagnostic media, a total of 400 plates are needed in comparison with 10,000 tubes of

media. A total of 1,000 isolates may be isolated, replicated, and categorized within 5 days.

Advantages of the replicator include the following: (i) construction is simple, and parts cost less than \$10.00; (ii) man-hours needed for replicating total about one-tenth of those required for inoculating tubes; (iii) isolate mortality is minimized, because only one transfer is needed before replication. The efficiency at-

tained permits the use of a broader range of differential media plus the time to study more organisms; a more comprehensive analysis can thereby be performed.

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