APPLIED MICROBIOLOGY, May 1971, p. 943

Vol. 21, No. 5
Printed in U.S.A.

Assay System for Bacteriocins

J. R. TAGG AND A. R. McGIVEN
Department of Pathology, Monash University, Melbourne, Australia

Received for publication 15 March 1971

Bacteriocin activity can be detected and assayed by a modification of the punch-hole method.

A variety of techniques have been used to determine the potency of bacteriocin preparations These have been based either upon broth dilution methods or the more frequently used plate assays. In the latter, the bacteriocin diffuses radially through an agar layer from circular cups cut into the gel or from containers or filter-paper discs placed on the surface of the medium.

In the present method, petri dishes are filled with an appropriate nutrient medium to a thickness of 5 mm, and, depending on the anticipated width of the inhibitory zones, various numbers of holes are punched out of the agar, by using a cork borer of 4 mm diameter. The base of each hole is sealed with a drop (0.05 ml) of melted nutrient agar, and then standardized quantities (1, 2, or 3 drops) of bacteriocin preparations are added to the appropriate wells.

The inoculated plates are incubated at 37 C for 1 to 2 hr to allow for diffusion of the bacteriocin into the medium. The agar is then loosened from the edge of the petri dish with a sterile spatula; the medium is inverted and prized away from the dish so that it falls into the lid, exposing the bottom surface of the agar. The use of glass petri dishes, rather than plastic, facilitates the removal of the gel.

After drying at 37 C for 2 hr the freshly exposed surface is inoculated by flooding with a log phase culture of the appropriate indicator organism. The plates are drained, dried, and then incubated with lids uppermost until zones of inhibition have clearly developed.

This technique has the advantage of allowing the bacteriocin to diffuse into the agar before the indicator strains are added. By inoculating the wells with broth cultures of various organisms, comparisons may be made between the production of bacteriocin by different strains growing under identical conditions (Fig. 1).

Kekessy and Piguet (1) have described a similar procedure for the detection of bacteriocin production by strains spotted on the surface of an agar medium before inverting and applying the indicator cultures to the other side. In the present method, growth is confined to a defined area so, if the wells are inoculated with the same number of organisms, a direct comparison may be made between the diameter of the zones of inhibition and the amount of bacteriocin produced by different strains. Another advantage of this method is its enhanced sensitivity since only a thin layer of agar is interposed between the producer cultures and the indicator lawn. This layer is sufficient to exclude inhibition due to phages which are nondiffusing entities.

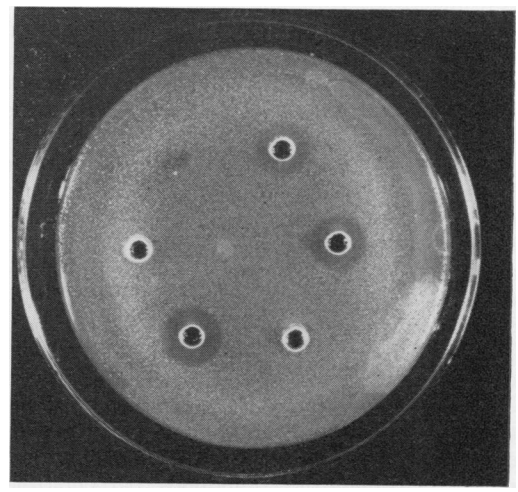

FIG. 1. *Bacteriocin inhibition of group A streptococcal indicator lawn on Todd-Hewitt medium by three producer strains of group A streptococci. Two strains are negative.*

We thank R. C. Nairn for advice.
This work was supported by a grant from the National Health and Medical Research Council.

LITERATURE CITED

1. Kekessy, D. A., and J. D. Piguet. 1970. New method for detecting bacteriocin production. Appl. Microbiol. 20:282–283.