MODIFICATION OF AN AGAR DIFFUSION METHOD OF ASSAY FOR POLYMYXIN

R. G. BENEDICT AND F. H. STODOLA

Fermentation Division, Northern Regional Research Laboratory[1], *Peoria, Illinois*

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Polymyxin is an antibiotic elaborated in the fermentation of various media by certain strains of *Bacillus polymyxa* (Stansly *et al.:* Bull. Johns Hopkins Hosp., **81,** 43; Benedict and Langlykke: J. Bact., **54,** 24). An agar diffusion method of assay was developed for the antibiotic (Stansly and Schlosser: J. Bact., **54,** 585). From a group of gram-negative organisms tested, *Escherichia coli* (MacLeod strain) was found to be the most satisfactory assay organism. However, its use presented certain difficulties since it grows very rapidly, and the antibiotic, which is incorporated in paper disks, diffuses slowly through agar. Thus it became necessary to incubate the assay plates at 25 C for 18 hours followed by incubation at 37 C for 6 hours to sharpen the edges of the zones of inhibition.

Using essentially the same assay techniques as those of Stansly and Schlosser, we have found that *Brucella bronchiseptica*, NRRL B-140, shows the same order of sensitivity to polymyxin as *Escherichia coli* (MacLeod strain). With *Brucella bronchiseptica*, the inhibition zones have very well-defined edges and are obtained by direct incubation of the assay plates at 37 C for 14 to 16 hours. Fermentation liquors also give sharply defined zones. For flooding plates the organism is grown in "trypticase soy" broth for 24 hours at 37 C, then 1.0 ml of undiluted broth is added to each 100 ml of flooding agar, mixed, and 4.0 ml of the mixture dispensed on each assay plate with an automatic agar dispenser. The plates, with covers slightly ajar, are then dried at 37 C in an incubator for 30 minutes. They are then removed and held at 20 C until the polymyxin-saturated disks are placed on them. Techniques for dilution of fermentation liquors and solid preparations are the same as those employed by Stansly and Schlosser except that the former are first diluted with equal quantities of 0.1 M glycine buffer in sterile 25-by-200-mm test tubes and further dilutions are made with 0.05 M buffer. Assay disks may then be saturated directly in these tubes and excess polymyxin removed by touching the disk twice to a dry surface in the upper part of the tube. The very slow rate of growth of *Brucella bronchiseptica* during the short 20 C holding period does not require that standard disks be placed on each assay plate. All unknown values are calculated from a daily standard curve. This curve is obtained by averaging the zones of inhibition on triplicate plates of 256, 128, 64, 32, and 16 polymyxin units per ml, as defined by Stansly and Schlosser (*loc. cit.*) and plotting them against the log concentration of polymyxin.

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.