How to prepare and use gradient plates.
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Background

Petri plates with a continuous gradient of concentrations from one side to the other were described by Szybalski and Bryson (1952). It was used by them to select bacterial mutants that had acquired increased resistance to antibiotics. It can be used to estimate tolerances and to obtain a semi-quantitative measure of resistance or response to a tested substance, and the method has seen wide applications.

Metzenberg and Grotelueschen (1992) prepared antibiotic gradients in square plastic petri dishes and streaked conidia from a specially designed heterokaryon to identify the phenotype of a sheltered component in which function of a gene is very suboptimal but is not completely absent.

Procedure

Two layers of agar are poured successively into a petri plate. The bottom layer consists of plain sorbose medium without the substance to be tested. The plate is propped up just enough for the agar to cover the entire bottom, forming a wedge that is shallow on one side and deep on the other. When the agar has solidified, the dish is placed in a horizontal position and another 10 ml of sorbose medium is added, this time containing the substance to be tested (3 to 10 times the estimated inhibitory or effective concentration). On incubation, downward diffusion of the drug or other substance results in its dilution proportional to the thickness ratio of the agar layers, establishing a uniform concentration gradient.

A suspension of conidia is streaked or spread over the surface.

References

Metzenberg, R. L., and J. S. Grotelueschen. 1992. Disruption of essential genes in Neurospora by RIP. Fungal Genet Newslett. 39: 37-49.

Szybalski, W., and V. Bryson. 1952. Genetic studies on microbial cross resistance to toxic agents. 1. Cross resistance of *Escherichia* coli to 15 antibiotics. J. Bacteriol. 64:489-499.

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