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Indicator plates for rapid detection of ribonuclease T1 secreting *Escherichia coli* clones

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Recently we constructed a Ribonuclease (RNase) T1 secreting *Escherichia coli* strain[1]. In analogy to a DNase test agar system[2,3] we now established a rapid screening assay for the detection of the mentioned overproducer on agar plates.

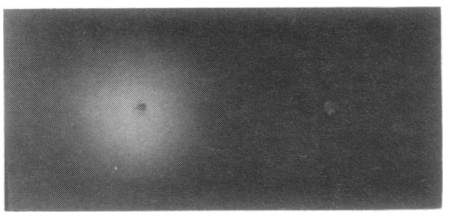

Fig. 1: Section of an RNase indicator-plate. After overnight incubation at 37°C an RNase secreting colony (left) showed pink halos against blue medium. An *E. coli* colony not secreting RNase is shown as a control (right). The test agar contained toluidine blue O (50 mg/l, Merck, Darmstadt; FRG) and yeast RNA (2 g/l, Boehringer, Mannheim; FRG) in solid LB medium (1.5 % Agar). RNA stock solution (pH adjusted to 7.0 using 1 N NaOH) was added at 50°C to autoclaved medium.

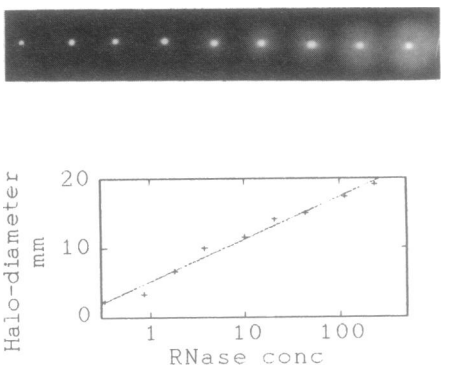

Fig. 2: Relation between halo diameter and the logarithm of the concentration of purified RNase T1. 0.25 µg-2.5 ng enzyme in 2.5 µl each were applied to holes of 2 mm diameter and incubated for 3 hours at 37°C. The detection limit is around 0.5 pmol for RNase T1 .

This assay is effective in screening a high number of mutant clones generated by site directed or random mutagenesis. It offers the advantage of picking selected clones directly from the toluidine blue O plates without replica plating.

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