

Deoxy ribonuclease (DNase) Test

Purpose

DNase Test Agar is used to distinguish *Serratia* species (+) from *Enterobacter* species, *Moraxella catarrhalis* (+) from *Neisseria* species, and *Staphylococcus aureus* (+) from other *Staphylococcus* species .

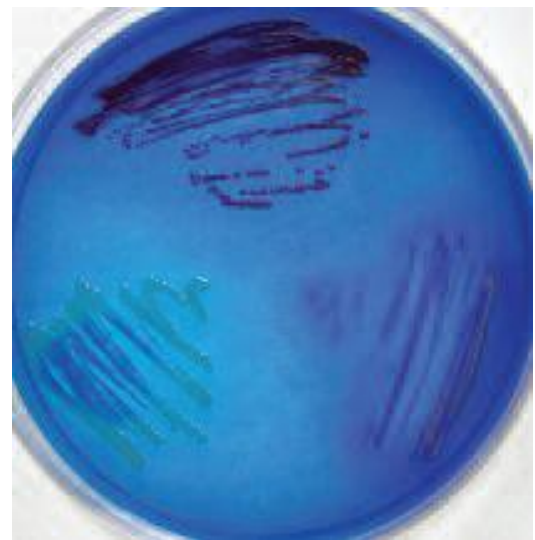
Principle

An enzyme that catalyzes the hydrolysis of DNA into small fragments (oligonucleotides) or single nucleotides is called **deoxy ribonuclease** or **DNase** . DNase is an **exoenzyme**, that is, an enzyme that is secreted by a cell and acts on the substrate extracellularly. This extracellular digestion allows utilization of a macro molecule too large to be transported into the cell. Ability to produce this enzyme can be determined by culturing and observing a suspected organism on a DNase Test Agar plate. Three versions of the test are available .One formulation for DNase Agar consists of peptides derived from soybean and casein that serve as carbon and nitrogen sources, sodium chloride for osmotic balance, and DNA as the substrate. After incubation, 1N HCl is added. Intact DNA will form a cloudy precipitate with the HCl, but not with nucleotides. Therefore, clearing around the growth is an indication of DNase activity (DNA hydrolysis) . A modification of DNase test agar includes toluidine blue , which forms a blue complex with intact DNA , but appears pinkish when complexed with nucleotides. DNase activity is indicated by a pink coloration around the growth . While this medium has the advantage of not using 1N HCl reagent, toluidine blue may inhibit some Gram-positive cocci, so it is recommended for use with *Enterobacteriaceae* .

An alternate modification of DNase Test Agar contains methyl green dye. The dye forms a complex with poly - merized DNA that gives the agar a blue-green color at pH 7.5 , but no complex is formed with nucleotides. Bacterial colonies that secrete DNase produce clearing around the growth of DNase positive organisms . The advantage to this medium is that adding 1N HCl is not necessary and it is appropriate for use with both Gram positive cocci and *Enterobacteriaceae*.



DNASE AGAR Clockwise from the top: *Staphylococcus aureus* (+), *Staphylococcus epidermidis* (-), *Serratia marcescens* (+), and *Enterobacter aerogenes* (-).



DNASE AGAR WITH TOLUIDINE BLUE Clockwise from the top: *Serratia marcescens* (+), *Staphylococcus aureus* (+), and *Enterobacter aerogenes* (-). *S. aureus* strains often grow poorly on this medium. For this reason, DNase plus TB is recommended for use with *Enterobacteriaceae*.



DNASE AGAR WITH METHYL GREEN Clockwise from the top: *Serratia marcescens* (+), *Enterobacter aerogenes* (-), and *Staphylococcus aureus* (+).