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Tributyrin Assays

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We use this protocol and it's working

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Abstract

Hydrolysis of lipids is an enzymatic process caused by extracellular lipases. To test production of lipases we use tributyrin, a triglyceride component, as a substrate.

Tributyryn Agar Test

- 1
 - Using a sterile inoculating loop, pick up a heavy inoculum from purity culture plate
 - Inoculate the tributyrin agar plate by drawing either a straight line or forming a circular inoculation over the surface
 - Incubate the plates at 37°C for about 24 to 48 hours aerobically for aerobes or facultative, and for about 72 hours anaerobically for anaerobes.
 - Following incubation, observe for formation of a clear zone of tributyrin hydrolysis around the bacterial colony
 - A *positive test* is indicated by the formation of a clear transparent zone of hydrolysis (tributyryn hydrolysis) around the bacterial colony (in the line).
 - A *negative test* is indicated by no clear zone of hydrolysis around the bacterial growth line (colonies).

Tributyryn Strip Test

- 2
 - Using a sterile inoculating loop, pick up a heavy inoculum from purity culture plate
 - Inoculate 25 mL of appropriate growth media and incubate at 37°C o/n
 - Using sterile forceps place one tributyrin strip into the bottom of a well. One per test organism
 - Add 30 µL of o/n growth onto the strip
 - For negative controls add 30 µL of PBS
 - For positive controls add 30 µL of o/n culture of *Staphylococcus epidermidis* strain VAD8.3
 - Incubate at 37°C and examine for colour change (pink to yellow) which indicates positive production of lipase

