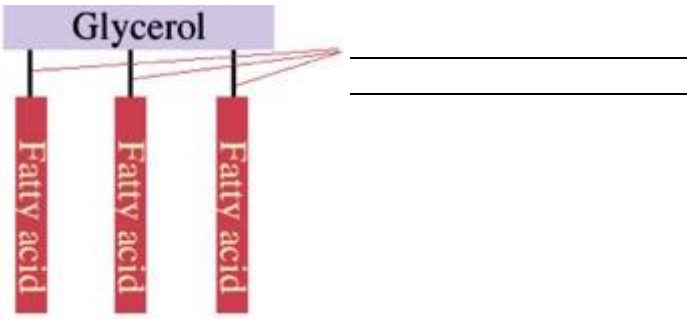
Lecture 19

I. Tests for gram-positive rods

A. Spirit Blue agar

1. This agar is used to identify organisms that are capable of producing the enzyme _____. This enzyme is secreted and _____ to _____ and three _____. These compounds are _____ to pass through the bacterial cell wall.



i. Glycerol can be converted into a _____.

ii. The fatty acids can be _____ and their fragments can eventually enter the _____.

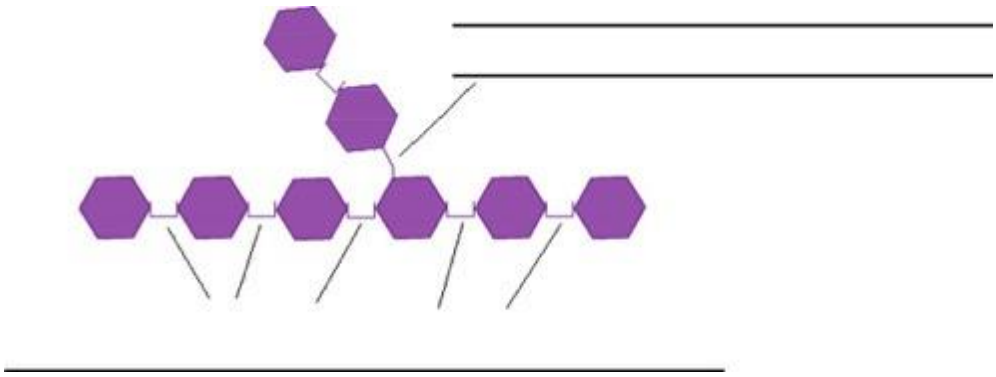
2. Spirit Blue agar contains an emulsion of _____. Bacteria that produce lipase will hydrolyze the olive oil and produce a _____ around the bacterial growth.

3. *Bacillus subtilis* is lipase _____.

B. Starch hydrolysis test

1. This test is used to identify bacteria that can _____ using the enzymes α-_____ and oligo-1,6-glucosidase.

2. Often used to differentiate species from the genera _____.



3. Because of the _____ of amylose and amylopectin molecules, these starches _____ through the bacterial cell wall.

i. In order to use these starches as a carbon source, bacteria must _____ α-amylase and oligo-1,6-glucosidase into the extracellular space.

ii. These enzymes _____ subunits which can then be utilized directly as carbon sources.

4. After incubation of the starch plates, _____ is added to the top of the agar. They iodine reacts with the starch to form a _____. Hydrolysis of the starch will _____ around the bacterial growth.

5. *Bacillus subtilis* is amylase _____.

II. Tests for gram-negative rods

A. Simmons Citrate Agar

1. This is a _____used to determine if an organism can use citrate as its _____. It is often used to _____

2. In citrate-positive organisms, the enzyme _____ hydrolyzes citrate into oxaloacetic acid and acetic acid. The oxaloacetic acid is then hydrolyzed into pyruvic acid and _____.

3. If CO_2 is produced, it reacts with components of the medium to produce an _____ (e.g. Na_2CO_3). The alkaline pH turns the _____ from _____. This is a _____ result.

4. *Escherichia coli* and *Shigella dysenteriae* are _____ whereas *Klebsiella pneumonia* and *Proteus mirabilis* are _____.

III. The Rapid KOH String Test[1]

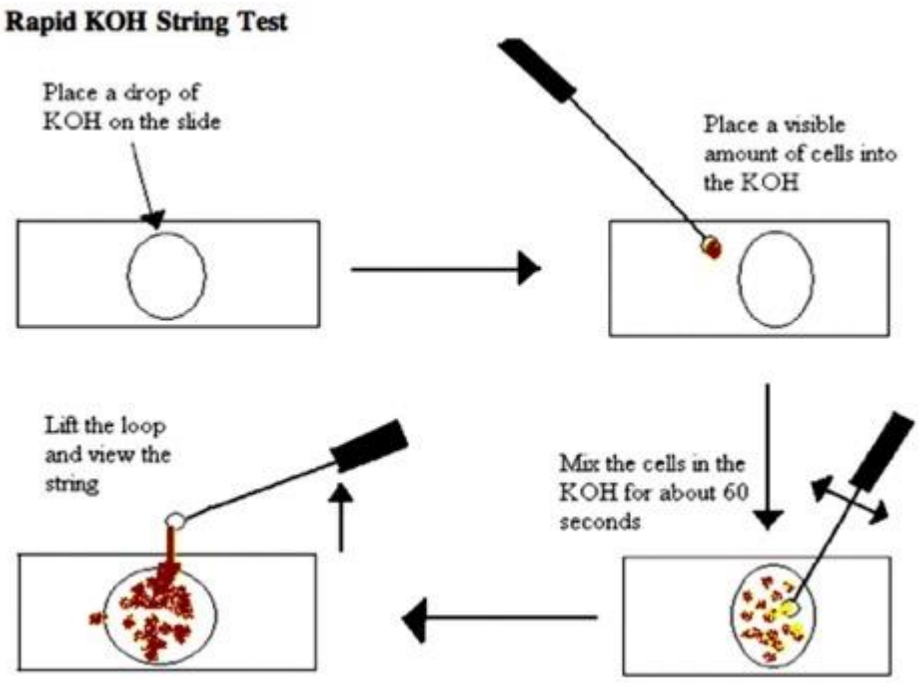
A. This test can be used in conjunction with the Gram stain to differentiate between Gram-positive and Gram-negative cells.

Check your knowledge: What are some potential problems with using the Gram stain alone to determine cell wall structure?

_____

_____

B. When placed in a drop of KOH base, Gram-negative cells will _____, releasing DNA and proteins into solution. A _____ of DNA and proteins is formed.[2] Gram-positive cells will remain clumpy in KOH.



1.This portion of the lecture was adapted from a procedure written by microbiology TA Aaron Larson in the spring of 2007.

2.Sutton, Scott. "The Gram Stain." The Microbiology Network. Feb 2006. Feb 20, 2007 <http://www.microbiol.org/WPaper.Gram.htm>