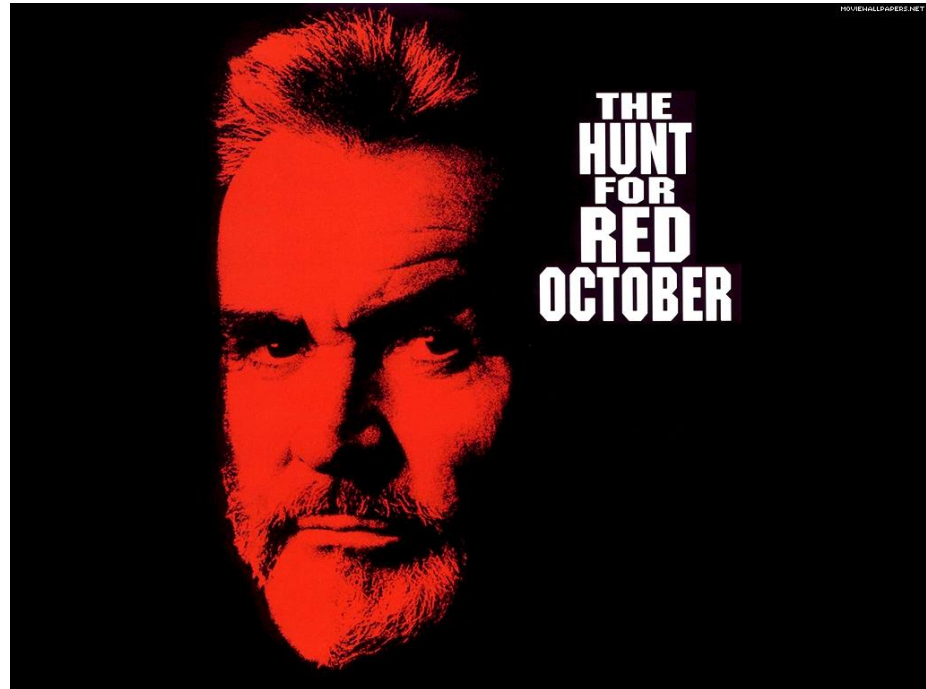


Metabolic Tests

Methyl Red (MR), Voges-Proskauer (VP)

- Methyl Red Test
- 15 drops Methyl Red to MRVP broth tube
- MIX
- Instant color change
- Methyl Red is a pH indicator
- Acid = red.



Methyl Red (MR), Voges-Proskauer (VP)

- VP Test
- Transfer 1 mL
- (2 people from each table with all tubes in rack)
- 9 drops VPA
- Mix
- 3 drops VPB
- Mix
- Wait 15-20 min
- Read every 10 min for 45 min
- Do not shake tubes.



Former VP, Dick Cheney



MRVP Test

- Methyl Red Test
 - Glucose fermentation → stable acids
 - Acid = red
 - (+) result
 - No acid = no color
 - Results
 - Record your data
 - *Enterobacter*, *Pseudomonas* (-)
 - *Escherichia coli* (+)
- VP Test
 - Glucose fermentation → acetoin + butanediol
 - After VPA and VPB
 - Red = (+) result
 - Don't confuse with copper color
 - Results
 - Record your data
 - *Enterobacter* (+)
 - *Escherichia coli* (-).



Figure 5-8

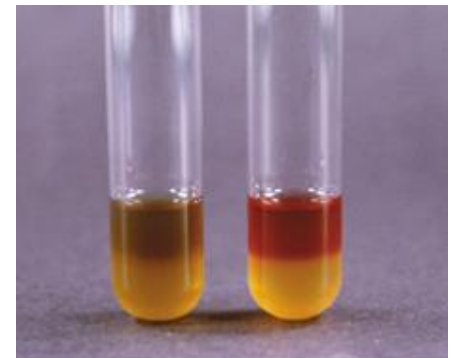
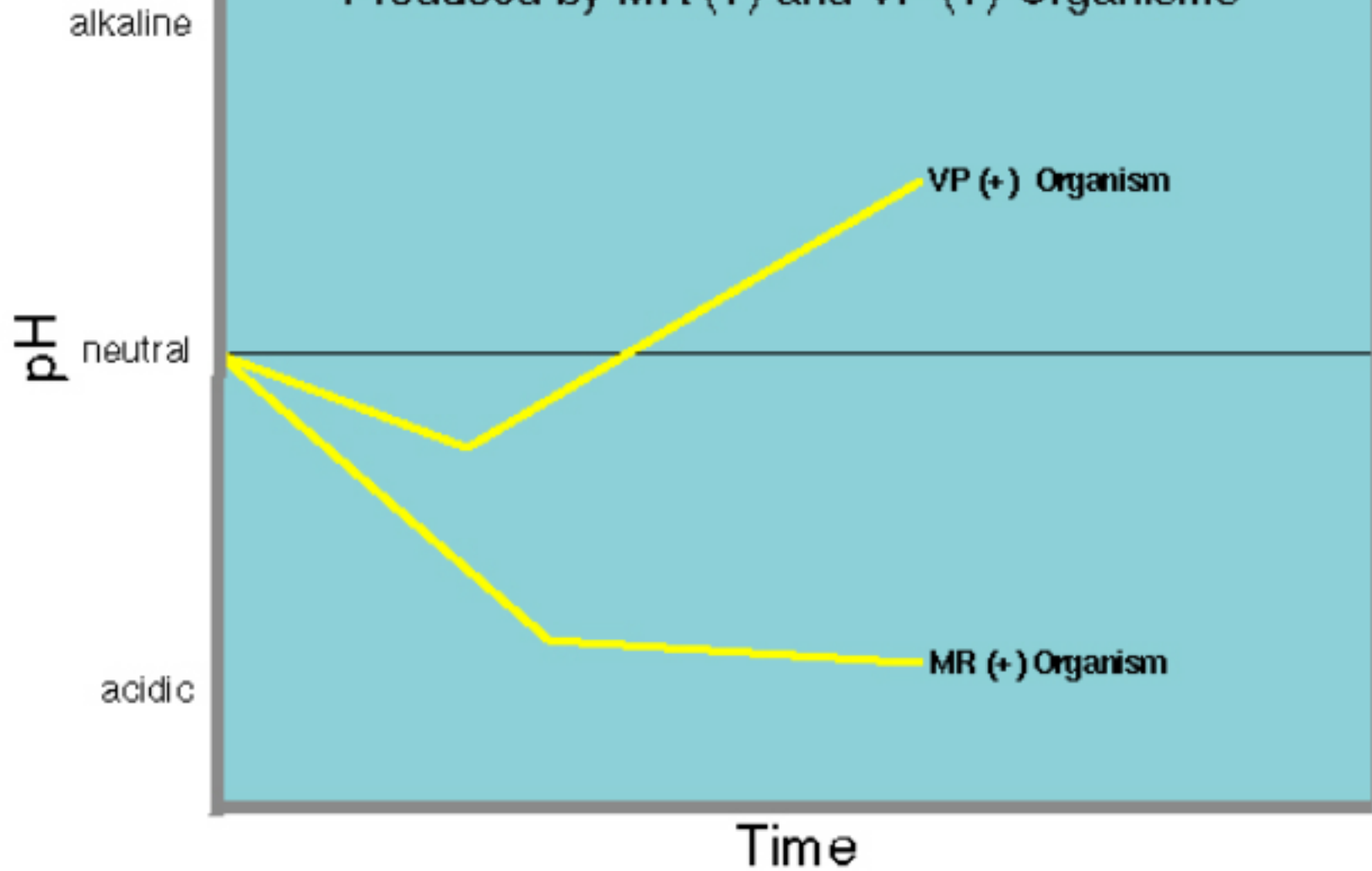


Figure 5-11

pH Changes in MR-VP Broth
Produced by MR (+) and VP (+) Organisms



Phenol Red (PR) Broth (Durham tubes)

- A simple broth that has a sugar in it
- The sugar can vary (use what you want)
- PR is a pH indicator
 - $\text{pH} < 6.8 \rightarrow \text{yellow}$
 - $6.9 < \text{pH} < 7.3 \rightarrow \text{red}$
 - $\text{pH} > 7.4 \rightarrow \text{pink/magenta}$



Figure 5-4

Phenol Red Broth

- Carbohydrate fermentation results in:
 - Acid end products
 - Acid end products + gas
- Gas will only be end product if there is also acid.



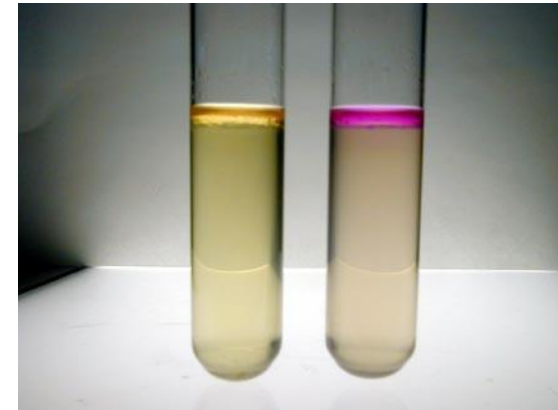
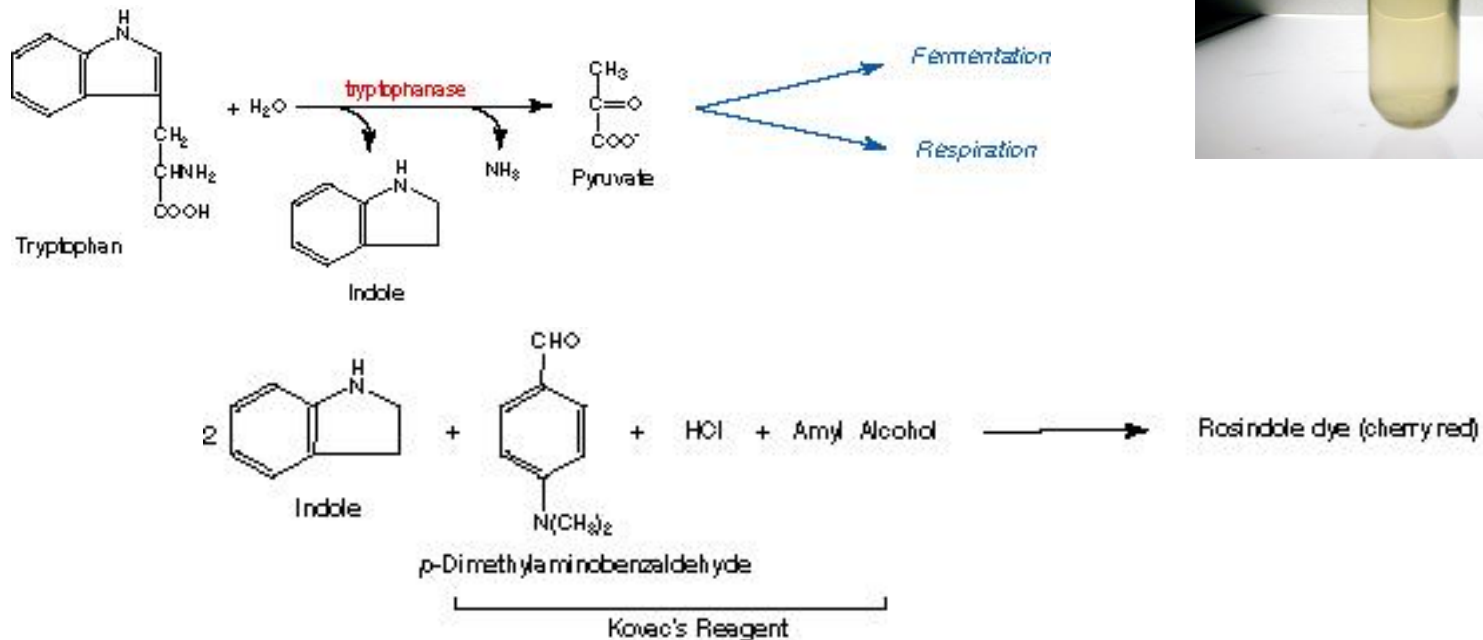
Results



Acid/gas: +/+ +/- C -/- -/- protein degradation

Indole Test

- 15 drops Kovac's Reagent
- Indole (+) = red
- Indole (-) = no color change.



Kligler Iron Agar

- Glucose
 - 1g in 1L
- Lactose & Sucrose
 - 10g each in 1L
- Phenol red
- Sodium thiosulfate
- Ferrous sulfate .



Kligler Iron Agar

- Glucose is preferred sugar
- But only 0.1%
 - So it runs out within 12 hours
- 0.1% glucose → acid (small amount)
- Then goes to alternate source
- beef extract/peptone → NH_3 (raises pH)
 - Reversion
- Slant reverts, but not the butt
- Glucose only fermenter results:
 - Basic/acidic, H_2S (+/-), gas (+/-)



Figure 5-68

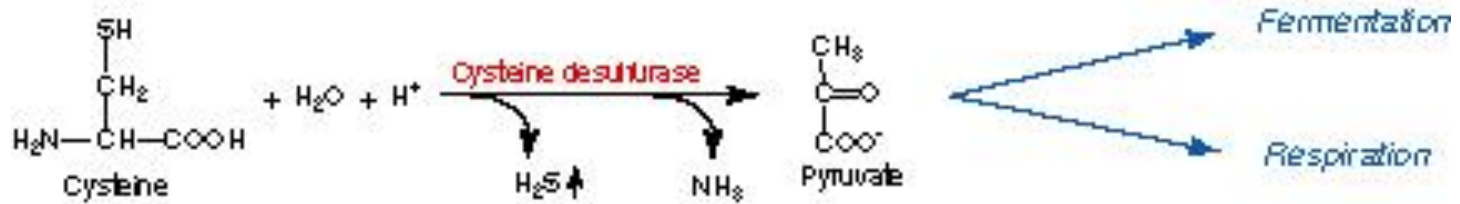
Kligler Iron Agar

- Glucose and lactose and/or sucrose fermenter
- 1% lactose, 1% sucrose (10x more than glucose)
- Lactose/sucrose → acid (large amounts)
- Tube is all yellow
- Acidic/Acidic, H_2S (+/-), gas (+/-).



Figure 5-68

Kligler Iron Agar



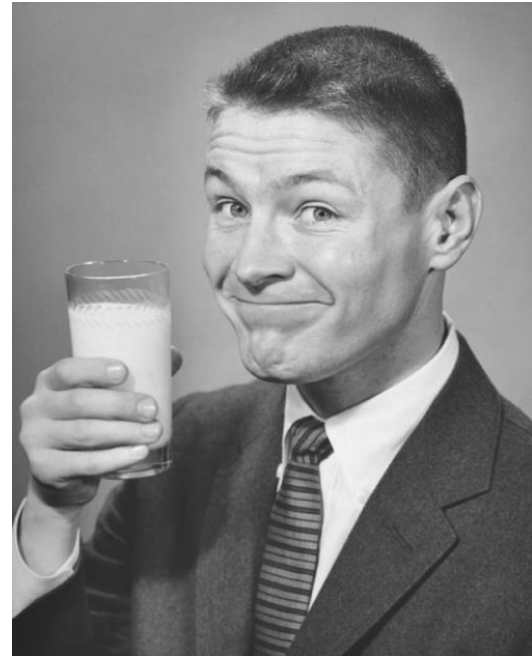
- Sugar fermentation produces acids (H^+)
- Cysteine Desulfurase works only in an acidic environment
 - Breakdown of Cys to pyruvate
 - Produces hydrogen sulfide (H_2S) gas
 - Pee-yeew!
- $\text{H}_2\text{S} + \text{Fe} \rightarrow \text{FeS}$ (ferric sulfide), a black precipitate
- Results
 - Any black is (+) for H_2S
- Unsure? Smell it!



Figure 5-63

Casein Hydrolysis Test

- Casein
- Protein found in milk
- Can be broken down by casease
- Bacteria get smaller peptides; source of amino acids.



Urease Test

- Urea is a nitrogen containing compound
- Urease is a hydrolytic exoenzyme
- NH_3 (ammonia) contributes to an alkaline pH
- Phenol Red
 - Acidic = yellow (-)
 - Neutral = red
 - Basic = hot pink (+)
- Please discard in specific waste bin.



Figure 5-50

EMB Agar

- Used to detect coliforms (fecal contamination)
- A selective medium
 - Encourages growth of Gram (-) organisms
 - Inhibits growth of Gram (+) organisms
- Ingredients include
 - Lactose
 - Color indicators: sodium sulfite and basic fuchsin
 - Detects changes in pH
 - Also double as Gram (+) inhibitors
- Inoculate with wavy streak.

EMB Agar

- Colorless or color of medium
 - Lactose nonfermenters
- Red with gold metallic sheen
 - Indicates vigorous lactose fermentation
 - Indicative of coliform bacteria (fecal contamination)..

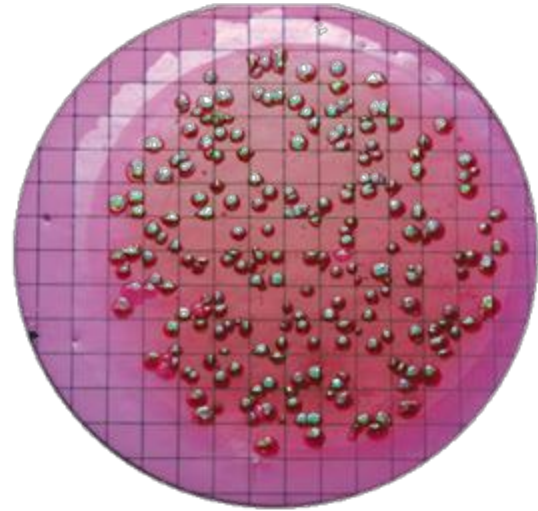


Figure 8-3



Toxic Buildup

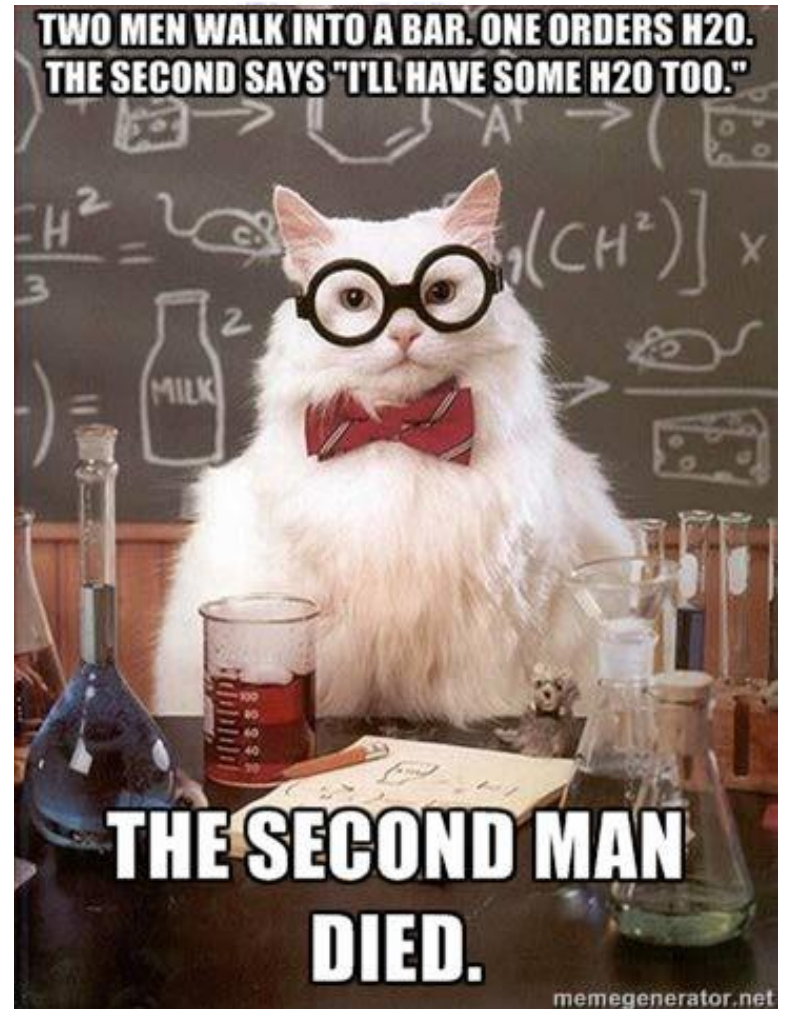
- The ETC can lead to a buildup of Hydrogen Peroxide (H_2O_2)
- $2 \text{H}_2\text{O}_2 \xrightarrow{\text{catalase}} 2\text{H}_2\text{O} + \text{O}_2$
- In order to engage in cellular respiration, bacteria need to make/have catalase.

Oxidase Test

- Take sterile cotton swab to pick up cells
- Apply 2 drops of oxidase reagent.
- Results
- Oxidase (+) = purple
- Within 30 seconds.

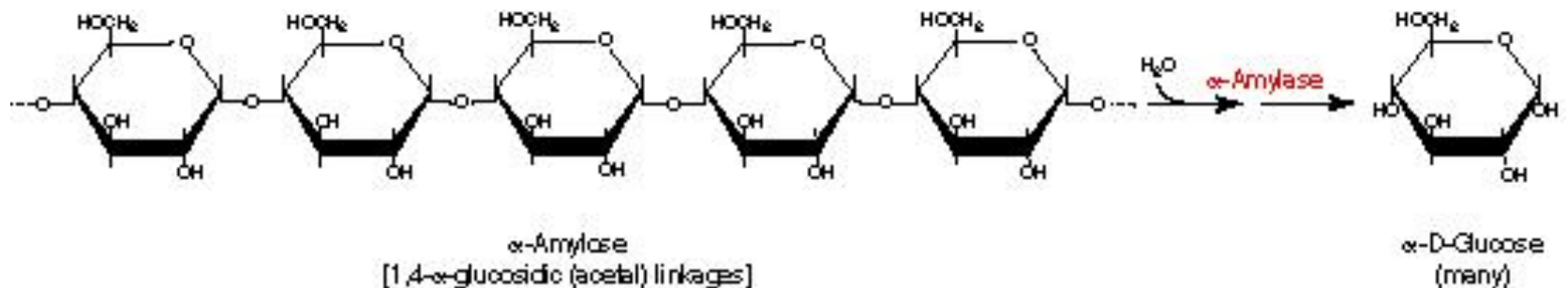
Catalase Test

- Use glass slide
- Use applicator stick to smear your cells
- Add 1-2 drops of H_2O_2
- Look for bubbling.



Starch Hydrolysis Test

- Starch is a polysaccharide
 - Of glucose monomers
- Some cells can breakdown starch to get glucose
- This requires special enzyme:.
 - Amylase.



Starch Hydrolysis Test

- To your streaked plate,
- Add iodine
 - Dark blue/black in presence of starch.



Figure 5-44

TABLE OF RESULTS		
Result	Interpretation	Symbol
Clearing around growth	Amylase is present	+
No clearing around growth	No amylase is present	-

TABLE 5-14 Amylase Test results and interpretations.

Spirit Blue

- Lipase (exoenzyme)
- Breaks down lipids
- Look for halos around colonies (transparent blue)



Citrate Test

- Used to differentiate organisms based on ability to grow when essential nutrient (like carbon) is limited
- Sodium citrate = sole C source
- Ammonium phosphate = sole N source
- Citrate → alkaline (NH_3) + ammonia products + Bromthymol blue
 - (blue when pH increases)



Figure 5-28

TABLE OF RESULTS		
Result	Interpretation	Symbol
Blue (even a small amount)	Citrate is utilized	+
No color change; growth	Citrate is utilized	+
No color change; no growth	Citrate is not utilized	-

TABLE 5-9 Citrate Test results and interpretations.

Mannitol Salt Agar

- Ingredients include:
 - Beef extract
 - Peptone
 - NaCl (75 g in 1L H₂O = 7.5%)
 - [salt] in bacteria cell = 0.85%)
 - Mannitol (fermentable; get acids)
 - Phenol Red (pH indicator)
 - Acidic = yellow
 - Neutral = red
 - Basic = hot pink
- Which ingredient is selective?
 - Which ingredient is differential?.

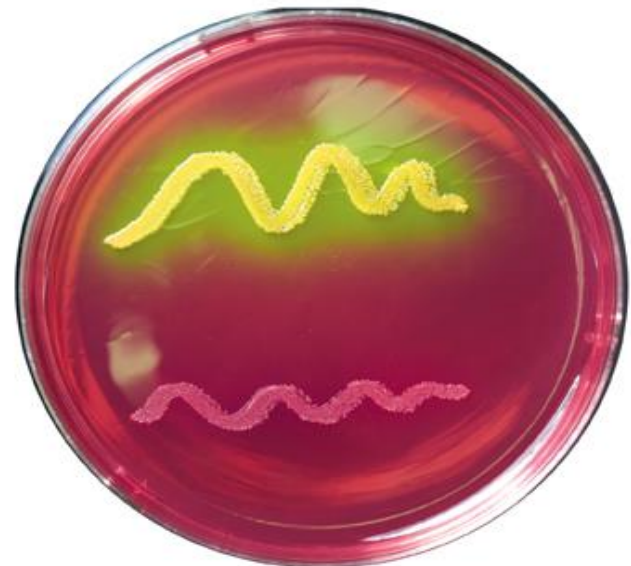


Figure 4-2

Mannitol Salt Agar

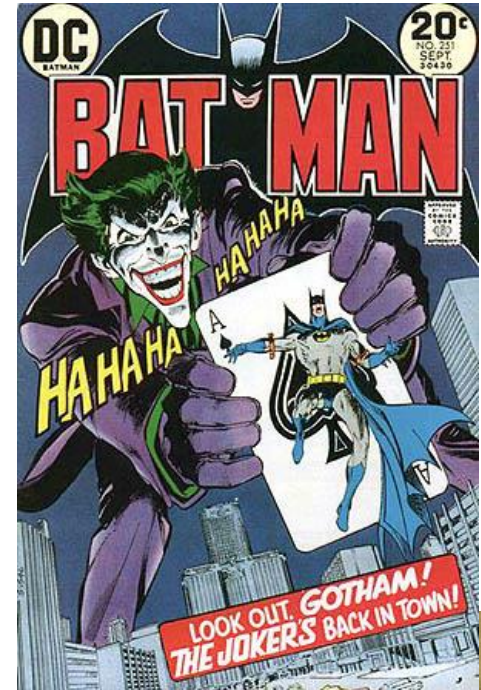
- A differential and selective medium
- Favors growth of *Staphylococcus*
- Differentiates:
 - pathogenic staphylococcus (ferments mannitol)
from nonpathogenic staphylococcus (does not
ferment mannitol).

Nitrate test

- Anaerobic respiration involving reduction of an inorganic molecule other than oxygen.

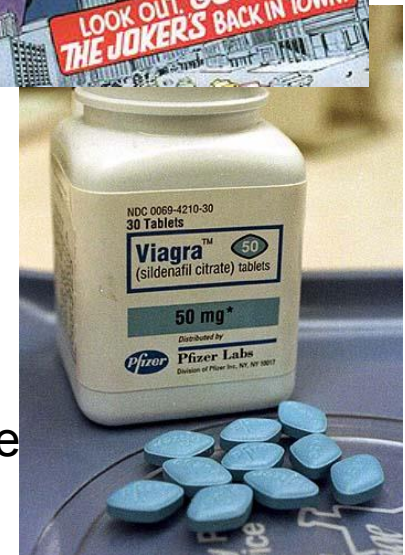


N_2O
Nitrous Oxide



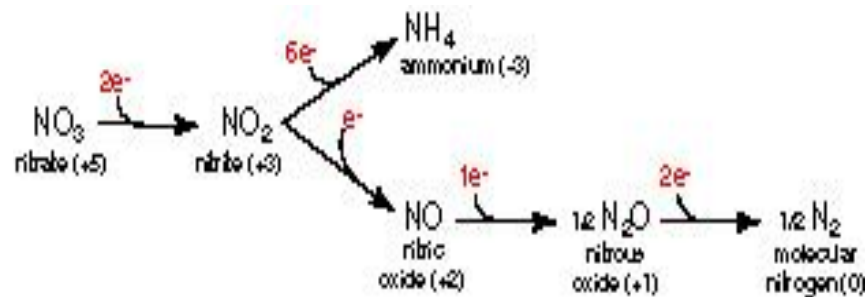
NO
Nitric oxide

"I need more NOS!"



Nitrate Reduction Broth

- Denitrification
 - Converting nitrate to N_2
 - This occurs when O_2 is not the final electron acceptor.



Nitrate Reduction Broth

- Add 6 drops of Nitrate Reagent A and B
- Within 2 min,
- Red = (+) for nitrite
 - (NO_3 was reduced to NO_2)
- If no red color, add a pinch of zinc powder
- Within 2 min,
- Zinc + NO_3 = Red
 - a (-) result for NO_3 reduction!
- If no red color develops after zinc,
 - (+) for NO_3 reduction.

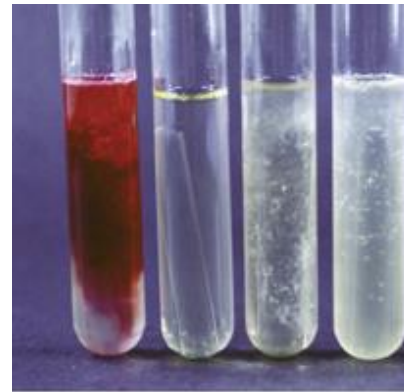
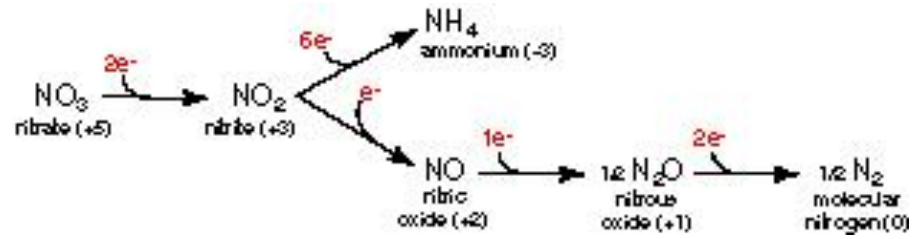


Figure 5-24

Reagent A & B added

Zinc added

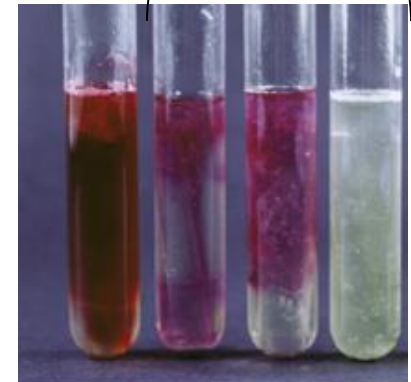


Figure 5-25