**Binder’s notes**

Micro II Lab midterm

**Carbohydrate Dehydration**

Permeases= transport proteins

Carbohydrases=endoenzymes (break down carbs)

**pH indicators**

* Phenol red: red pH 6.9 or higher; yellow pH 6.8 or lower
* Methyl red: red pH 4.4; yellow at pH 6.0
* Bromthymol blue: green pH neutral; blue pH 7.6 or greater; yellow at pH acidic

**Simmons citrate**: used to identify organisms that utilize citrate for energy; medium turns from green (pH 6.9) to blue (pH > 7.6) when positive.

* **E.coli: Negative**
* **B. Subtilis: Positive**

**Triple Sugar Iron (TSI):** used to identify Gram-neg enteric rods. Measures bacteria’s ability to utilize glucose (0.1%), sucrose (1.0%) and lactose (1.0%). Slant and stab used. Glucose utilization only=yellow in “butt” (bottom of tube); sucrose or lactose= yellow through whole medium.

* Yellow color change= positive results; acid production; carb utilization
* Black/brown color= positive for hydrogen sulfide (reduced from sodium thiosulfate)
* Cracks in agar= positive for other gases produced in agar
	+ **E.coli: yellow color throughout agar**
	+ **B. subtilis: yellow color on top**

**Starch Hydrolysis:** identifies organisms that can break down starch. Organisms containing **amylase** and **oligo-1,6-glucosidase**. Hydrolysis of starch turns agar clear.

* **E.coli= negative**
* **B.subtilis= positive (clear around colony**

**Catalase and oxidase:** enzymes related to an organisms ability to utilize oxygen

 **Catalase**: degrades hydrogen peroxide formed when oxygen and water are metabolized

* Only found in **aerobes**
* Bubbles with hydrogen peroxide=**positive catalase**
* **Streptococcus faecalis= negative** for catalase
* **Staphylococcus aureus= positive** for catalase

**Oxidase:** catalyzes the transport of electrons from a donor compound (phenylenediamin) to the final electron acceptor, oxygen. **Blue/purple color is positive for oxidase**. No color change is negative for oxidase.

* **E.coli= negative oxidase**
* **Pseudomonas aeruginosa= positive oxidase**

 **Nitrate Reduction:** testing for nitrate reductase or nitrite reductase.

 Nitrate broth🡪nitrate reductase🡪Nitrite🡪nitrite reductase🡪no n2 (denitrification)

 Durham tube used to test denitrification; **no color=denitrification**

 Nitrite: 5 drops nitrate reagent; **red color=positive for nitrite**

 Nitrate: zinc and 5 drops 6M HCl; **red color=positive for nitrate**

* **b.subtilis= red, positive nitrate**
* **staph aureus= red, positive nitrite**
* **pseudomonas aeruginosa= no color; denitrification**
* **Dirt= no color; denitrification**

**DNAse:** enzyme digests DNA into constituent nucleotides.

**Intact DNA**=cloudy (negative).

**hydrolysis of DNA**= clear (positive)

**Indole:** tryptophan medium inoculated; tested for indole

 **Tryptophanase**: breaks down tryptophan into indole, pyruvic acid and ammonia

 **Kovacs reagent**: produces red color if Indole present (positive results)

**Urease:** enzyme that breaks down urea into ammonia and co2; broth contains urea and phenol red

 **Purple/fuschia color**: positive for Urease; urea digested and pH of broth raised

|  |  |  |  |
| --- | --- | --- | --- |
|   | DNAse | Indole | Urease |
| Staph aureus | + | - | - |
| K. pneumoniae | - | - | + |
| e.coli | - | + | - |

**Hemolysis:** Blood agar with sheep or rabbit blood. Differential medium used to differentiate bacteria on their ability to break down (lyse) blood in the media.

* **Alpha:** partially destroys hemoglobin; causes greenish cloudy area around colony
* **Beta:** Completely destroys hemoglobin; clear zone around the color
* **Gamma:** non-hemolytic; no change in media
	+ **Staph Aureus= Beta (complete RBC destruction)**
	+ **E.coli= gamma (no change in media)**
	+ **Strep. Faecalis= alpha (partial, greenish)**

\*\*\*\*Read through all the introductions for further details\*\*\*\* Jeffbinder@gmail.com for questions