**Microbiology Lab Final Study Guide**

1. Anatomy & Classification of Bacteria

Shape:

1. Bacillus – rod
2. Coccus – spherical
3. Corkscrew or spiral – spirochete / spirrillum
4. Caulobacterium – stalked
5. Corynebacterium – clubbed form
6. Comma shape – vibrio

Arrangement:

1. Staphylo – clusters
2. Strepto – chains
3. Diplo – pairs
4. Micro – single
5. Group of four – Gaffkya
6. Cube of eight – sarcina

|  |  |  |
| --- | --- | --- |
| NAME | SHAPE / ARRANGMENT | GRAM STAIN |
| STRETPTOCOCCUS | COCCI, CHAIN | **(+)** PURPLE; Thick layer of peptidoglycans – Aas & sugars; contain original dye |
| STAPHYLOCOCCUS AREUS | COCCI, CLUSTER (GRAPES) | +  |
| M. LUTEUS | COCCI, SINGLE | + |
| CLOSTRIDIUM SPOROGENES | ROD, SINGLE | + |
| BACILUS AREUS | ROD, CHAIN | + |
| E. COLI | ROD, SINGLE | **(-)** PINK; Thin peptidoglycan layer surrounded by outer layer of protein and liposaccharides; get decolorized |
| PSEUDOMONAS FLURESCENS | ROD, SINGLE | (-) |
| PSEUDOMONAS AERUGINOSA | ROD, SINGLE | (-) |
| ACINETOBACTER CALCOACETICUS | ROD, PAIR | (-) |

1. Differential Staining: The Gram Stain

Intro:

* Differential staining = method of distinguishing between different types of bacteria

Gram Stain:

* Most common and useful differential stain
* Divides bacteria into two stains (-/+)
* Different cell wall structures have different permeability to dyes used (SEE 1st exp)
* **4 Solutions required**
	+ Basic dye – *Gram’s crystal violet*
	+ Mordant (helps fix dye on cell) - *Iodine*
	+ Decolorizing agent (removes dye from stained cells – some decolorize easier) *Acetone-alochol*
	+ Counter stain (makes decolorized bacteria visible) – *Safranin*
* Gram positive: contain original dye; Gram negative: get decolorized

Procedure:

* Smears – E Coli, Bacilus cereus, Staph aureus & mixed E Coli / Bacilus areus
* Heat fix, crystal violet (1 minute), water, iodine (45-60 seconds), water, decolorizing solution (stop when color is gone), safranin (30-60), water / dry
1. Aseptic Technique (E. Coli & M. Luteus)

Intro:

* Pure culture – contain only one species
* Contaminated – not pure; result from not following aseptic technique
* Aseptic technique = requires use of materials free of contamination; goal is to maintain pure cultures

Culturing organisms:

* Cells (inoculums) transferred (inoculated) into sterile medium:
	+ Liquid – culture tube; LARGE quantities of bacteria
	+ Solid – Agar slants (stab, smear)
* Consider for optimum growth: temp (37 degrees for plates), pH, O2 tension, hydro pressure and radiation
* Methods to describe way in which culture grows:
	+ Turbidity – cells suspended
	+ Pellicle formation – cells float on top
	+ Sediment – cells rest at bottom

Aseptic technique procedures

* GENERAL
	+ Location: free of traffic, air currents
	+ Contamination: Wear lab coat or apron; clean work station before and after
	+ Tools: inoculation loop – hold at 60 degrees; heat loop from handle to tip of loop
* LIQUID Culture transfers
1. Heat sterilize loop
2. Remove closures from tubes
3. Flame open ends of tubes
4. Insert loop into bacterial culture
5. Insert loop into sterile broth
6. Replace closures
7. Heat sterilize loop
* SOLID Culture transfers
1. – 3) Same as above

4) Inoculating slant: zig-zag pattern; stab technique

5) Inoculating plate *(pure cultures only):* three section zig-zag pattern

\*\*\* Solid and liquid cultures can be inoculated using either kind of culture\*\*\*

1. Free Oxygen

Intro:

* Gaseous oxygen requirement:
	+ Strict aerobe – requires Oxygen; grows only on top of agar
	+ Strict (obligate) anaerobe – oxygen is toxic; will grow on bottom of test tube
	+ Facultative anaerobe – can grow in either presence or absence of Oxygen (better in O2); grow throughout media
	+ Microaerophiles – does well in reduced-oxygen conditions, still require some; grow on surface or just below
* Bacteria: E.Coli, Clostridium sporogenes, M. Luteus
* YTA = Yeast-extract Tryptone Agar tubes
* BCP-G = Brom Cresol Purple-glucose tubes (purple – alkaline pH; yellow – acidic pH: bacteria use up media to produce acid)

Procedure:

* Cooled tubes to 45 C
* Incubated at 37 C until next lab period

Results:

|  |  |  |
| --- | --- | --- |
| BACTERIA | O2 REQUIREMENTS | YTG / BCP-G |
| E.Coli | Facultative anaerobe | Throughout |
| Clostridium Sporogenes | Strict (obligate) anaerobe | On bottom |
| M. Luteus | Strict aerobe | On top |

1. Motility

Intro:

* Motility – helps bacteria escape harmful substances and acquire food by growing beyond stab line
	+ Prokaryotes – gliding or w/ flagella
	+ Small, non-motile bacteria: Brownian movement – random movement in different directions due to bombardment of the cell with water molecules
* Methods of determining motility
	+ Direct observations of live cells under microscope w/ hanging drop
	+ Stab inoculating soft agar medium, allowing mobile bacteria to grow beyond stab line
* Bacteria: Pseudomonas aeruginosa (- Rod) & Staph aureus (+ grape cluster)
* Motility test agar

Results:

* Pseudomonas aeruginosa: + Motility (grew beyond stab line)
* Staph aureus: - Motility
1. Temperature

Intro:

* Microbes found in almost every environment
	+ Easily dispersed
	+ Need only small quantities of nutrients
	+ Diverse nutritional requirements
	+ Great capacity for adaptation
* Microbial habitats defined by physical environment and nutritional needs
	+ Physical factors:
		- pH, Temp, O, moisture, hydro pressure & radiation
	+ Nutritional factors
		- C, N, S, P & vitamins
* Bacteria can be catalogued by their temp requirements
	+ Psychrophiles – like; require temps of 0-20 C
	+ Mesophiles – require temps of 21-40 C
	+ Thermophiles – like; require temps of 41-100 C
	+ Microbes --- can tolerate 30C range
		- Obligate – strict w/ temp requirements
		- Facultative – less strict
* Growth temp defines organism’s temp range
	+ Maxiumum growth temp – highest temp at which cell division can occur
	+ Optimum growth temp – fastest cell division occurs
	+ Growth gradually increases with increasing temp and when optimum temp is close to max temp
	+ After max temp – cell division decreases
* Above freezing point: Enzyme activity doubles with every 10 C increase in temp until denaturation occurs
* Bacteria: E. Coli, M. Luteus, Bacillus subtilis & Pseudomonas fluorescens (- rod)
1. pH

Intro:

* pH requirements:
	+ Acidophiles: like pH 1-5; organisms that produce acid from cell resp
	+ Neutrophiles: like pH 5.5-8.4
	+ Alkalphiles: like pH 8.5-11.5; decomposers
	+ Generally, microbes grow best at pH similar to human blood (7.4)
* Bacteria: E.Coli, M. Luteus, Bacillus subtilis & pseudomonas fluorescens

Procedure:

* Mark off four quadrants on bottom of each adjusted pH plate: 5, 7, & 9
* Streak one organism on same quadrant for each plate
* Incubate tubes at 37 C for 24 hours

Results: Most growth in pH 7

1. Bacterial Transformation

Intro:

* Ability to acquire new traits favors survival and enhances diversity
	+ Mechanisms of gene transfer – transformations used by genetic engineers
* Bacterial transformation: DNA (plasmid or chromosomal) is transferred from a donor to a recipient cell as an unprotected piece of naked DNA
	+ Donor cells lysed first to allow for DNA release
	+ Following release, chromosomal large segment broken into smaller naked DNA frags; plasmid DNA remains in tact
* Competent cells = cells capable of taking up high-molecular weight DNA are said to be competent; Acinetobacter are naturally competent
* Two methods of bacterial transformation:
1. Ionic detergent
	* + Sodium dodecyl sulfate (SDS) to prepare extract of streptomycin-resistant (Str-r) culture of Acinetobacter calcoaceticus
		+ Acin Calc used to transform naturally competent streptomycin-sensitive (Str-s) strain of A. Calc into one able to grow in media containing streptomycin
		+ **Susceptible strain won’t grow on stepto plate**
2. “Heat-shock” transformation
* Bacteria unable to uptake DNA = competent
* Treat with CaCl in early phase of growth
* Heat-shock genes = set of genes which aid bacteria in surviving at higher temps (E. Coli @ 42 C)
* Heat-shock step is necessary for uptake of DNA

To help cells recover, they are incubated with non-selective growth media.

Due to low number of bacteria cells which contain the plasmid and the potential for the plasmid not to propogate itself in all daughter cells, it is necessary to select for bacterial cells that contain the plasmid

Definitions:

* Transformation – the uptake by a recipient bacterium of a naked DNA molecule or a fragment from a culture medium and the incorporation of this molecule into the recipient chromosome in a heritable form
* Competent cell – a bacterium able to take up DNA and be transformed
* Auxotroph – mutated bacterium that lacks the ability to synthesize an essential nutrient and must obtain it from its surroundings

Materials:

* BHI = Blood Heart Infusion
* BHI agar plate of wild type Str-s AC (susceptible strain – killed by strepto)
* BHI agar plate of stable Str-r AC (resistant strain)
* SDS = Sodium Dodecyl Sulfate detergent
* Streptomycin – antibiotic

Results / Drawings:

1. Antibiotics

Intro:

* Antibiotics are chemical agents that are produced by living organisms that can kill or inhibit growth of other organisms
* When a clinically significant microorganism is isolated from a patient, the most effective agent or more important, the antibiotics, which would be ineffective, can be determined by a simple lab analysis: Kirby-Bauer test
* Kirby Bauer test
	+ Paper disk is saturated with antimicrobial agent (antibiotic) and placed on petri dish that had been incoculated with test organism
	+ Antibiotic can diffuse from the disk into the surrounding medium.
	+ Zone of growth inhibition can be measured
		- Wider the circle, the more sensitive the organism
		- Test determines whether the organism is resistant, sensitive, or intermediate
		- **Measured from back side of disk across center, to edge of plaque**

Results:

* E. Coli: No growth
* Pseudo: 20mm (susceptible)
* Sacch: 20mm (susceptible)
* Staph: 19 mm (susceptible)
1. Normal Flora

Intro:

* Under normal conditions, human throat and mouth contain considerable numbers of microorganisms
* Oragnisms isolated from healthy individuals:
	+ Staph species
	+ Strepto species
	+ Etc….
* Viridians group
	+ Streptococci
	+ Produce Alpha hemolysis (PARTIAL clearing of RBCs – greenish, cloudy area)
	+ Most prominent of throat bacteria
* Blood agar used for cultivation
* Beta hemolysis – completely destroys RBS; decolorizes the hemoglobin (clear zone)
* Gamma hemolysis (non-hemolytic): no effect on RBCs