Microbiology for the Health Sciences Laboratory

Lab Sessions 7 - Microbial Physiology

Part I: Selective & Differential Media, and Hydrolytic Enzymes
Part II: Oxidation-Reduction Reactions, and Rapid, Multitest Systems

Introduction

Isolation and identification of microorganisms is based primarily on their specific metabolic characteristics. Selective and differential media are used to help us isolate and tentatively identify organisms. Various culture media are then employed to determine specific metabolic properties of isolated organisms based on their unique biochemistry. We then use a combination of morphological and physiological determinants to positively identify an isolated, unknown organism. The purpose of these two laboratories is to perform many of the most common isolation and identification procedures used in microbiology labs.

A. Review the following for reference:

1. Text: Chapter 4 - Pages 94-97, and Chapter 10 – pages 241-245.

B. Do the following during lab:

1. Use the attached list as a guide for which microorganisms to inoculate into which media.
2. Work in groups. Divide culture plates as needed for inoculations. Label the cultures well!
3. Incubate the cultures for 48 hours at 37 C.
4. Note: You should treat all organisms as if they were BSL-2 mo's. However, actual BSL-2 mo's are labeled with an *
   Salmonella sp and *Shigella* sp can only be transferred with my direct supervision!

C. Techniques:

Follow the directions for the type of inoculation:

Isolated colonies
(Use only half the plate to do the isolation!)

Smear	Spot	Stab
Use an inoculating loop to make a large inoculum	Use an inoculating loop to make a small inoculum	Use an inoculating needle to 'stab' the 'deep'

D. Inoculations and Results. **Part I**: **Selective & Differential Media, and Hydrolytic Enzymes**

Culture Media	Form	Type	Inoc.	M.O.'s	Results/Reactions
Blood Agar	Plate	Differential	Isolated Colonies	*Streptococcus pyogenes**	
				Staphylococcus epidermidis	
				Streptococcus salivarious	
				*Staphylococcus aureus**	
				Neisseria sicca	
				Candida albicans	
		Alpha-Hemolysis = Greening, e.g. *Strep. pneumoniae;* Beta-Hemolysis = Clearing, e.g. *Strep. pyogenes;* Gamma-Hemolysis = No hemolysis, e.g. *Staph. epidermidis*			
Columbia Agar	Plate	Selective	Smear	*Staphylococcus epidermidis*	
				*Staphylococcus aureus**	
				Escherichia coli	
				Corynebacterium xerosis	
		Colistin and Naladixic acid select for gram (+) mo's			
Mannitol Salt Agar	Plate	Sel & Diff	Smear	*S. epidermidis*	
				*S. aureus**	
				Serratia marcescens	
				E. coli	
		7.5% NaCl is selective for *Staph*. Sp. MSA is differential for *S. aureus* due to fermentation of mannitol (= Yellow)			
Eosin Methylene Blue Agar	Plate	Sel & Dif	Smear	*E. coli*	
				*Pseudomonas aeruginosa**	
				Enterobacter aerogenes	
				S. epidermidis	
		Dyes (eosin and methylene blue) select for gram (-) mo's. *E. aerogenes* = Pink, *E. coli* = Green (Lactose fermentation), *Pseudomonas* sp = Clear.			

Culture Media	Form	Type	Inoc.	M.O.'s	Results/Reactions

Hektoen Agar	Plate	Sel & Dif	Smear	*E. coli* *E. aerogenes* *Salmonella enteriditis** *P. vulgaris**

Selective for enteric pathogens.
Shigella sp. = Green
Salmonella. sp. & *Prot*eus sp. = Blue-green & black
Enterobacter sp. & *E. coli* = Salmon pink to orange.

Tellurite Glycine Agar	Plate	Sel & Dif	Smear	*E. coli* *S. epidermidis* *S. aureus** *Corynebacterium xerosis*

Selective for coagulase (+) staphylococci. Black = Coagulase production.
Gray = Non-pathogenic *Staphylococcus* sp. and *Corynebacterium* sp.

Potato Dextrose Agar	Plate	Selective	Smear	*S. epidermidis* *E. coli* *Saccharomyces cerevisciae* *Candida albicans*

This is a selective medium (due to increased sugar concentration) for the isolation of fungi.

Mycobiotic Agar	Plate	Selective	Smear	*C. albicans* *S. epidermidis* *E. coli* *S. cerevisciae*

This is a selective medium for the isolation of pathogenic fungi.
It contains the antibiotics cycloheximide and chloramphenicol.

Culture Media	Form	Type	Inoc.	M.O.'s	Results/Reactions
Starch Agar	Plate	Hydrolysis	Spot	*Bacillus subtilis*^	

E. coli

S. epidermidis

B. cereus^

Tests for production of an extracellular enzyme that degrades polysaccharides.
+ Test: Add iodine and look for clearing around colony.
^ Put this mo on a plate by itself.

Skim Milk Agar Plate Hydrolysis Spot *B. subtilis*^

E. coli

S. epidermidis

B. cereus^

Tests for production of an extracellular enzyme that degrades casein (i.e. milk protein).
+ Test: Clearing of media around colony.
^ Put this mo on a plate by itself.

Spirit Blue Agar Plate Hydrolysis Spot *S. aureus**

*E. aerogenes** .

E. coli

S. epidermidis

Tests for production of an extracellular enzyme that degrades lipids.
+ Test: Clearing of media (not increased color!) around colony..

DNase Agar Plate Hydrolysis Spot *S. aureus**

E. aerogenes *

S. epidermidis

*S. pyogenes**

Production of an extracellular enzyme that degrades DNA.
+ Test: Clearing of media around colony.

Part II: Oxidation-Reduction Reactions, and Rapid, Multitest Systems

Culture Media	Form	Type	Inoc.	M.O.'s	Results/Reactions
Oxidase	Plate	Redox	Spot	*N. sicca*	
				E. coli	.
				*P. aeruginosa**	
				S. epidermidis	
				Tests for production of oxidase. Add oxidase disc to growth on BHIA plate.	
				+ Test for oxidase: Disc turns purple to black.	
Catalase	Plate	Redox	Spot	*S. salivarious*	
				*S. aureus**	
				S. epidermidis	
				*S. pyogenes**	
				Tests for production of catalase. Add H_2O_2 to growth on BHIA plate.	
				+ Test for catalase: Bubbles	
Methyl Red	Broth	Redox	Loop	*E. coli*	
				E. aerogenes	
				Tests for mixed acid fermentation. Add several drops of methyl red to tube.	
				+ Test for acid (pH <5) production: Red color develops at interface	
V-P	Broth	Redox	Loop	*E. coli*	
				E. aerogenes	
				Tests for neutral product fermentation. Add Baritt's reagent A and B to tube. Mix well.	
				+ Test: Red color develops at interface. - Test: Yellow or brown color.	

Carbohydrate Fermentation Broths					Glucose	Sucrose	Mannitol	Lactose
	Broth^	Fermentation	Loop	*E. aerogenes*				
				E. coli				

Citrobacter freundii

*P. vulgaris**

S. marcescens

Interpretation of Carbohydrate Fermentation Broths:

Yellow = Acid production = A	Pink = Basic products = B
Yellow & Gas = Acid & gas = AG	Red & Gas = Alcoholic fermentation = G
Growth but no changes = No fermentation = +	No growth = -

^Phenol red broth with Durham tubes (to catch gas). Phenol red is red above pH 7.4, it changes from red to yellow between pH 7.4 and 6.8, and it is yellow below pH 6.8.

Kligar Iron Agar

				Butt	Slant	Gas	H_2S
Slant	Fermentation	Stab	*E. aerogenes*				
		and	*E. coli*				
		Swab	*P. vulgaris**				
			C. freundii				
			S. marcescens				

Interpretation of Kligar Iron Agar Slants:

Yellow butt/red slant (YB/RS) = glucose fermentation	Yellow butt & slant (YB/YS) = lactose/sucrose fermentation
Splitting of agar = gas production = G	Blackening = H_2S production = HS

Enterotube^

Multiple tests Injection

Requirements
Gram negative,
Oxidase negative,
Glucose positive,
Rods.

Examples:
E. aerogenes
*P. vulgaris**
S. marcescens
C. freundii
*S. enteriditis**
E. coli

Chamber # - Test for...	+ Test Result
1 - Glucose fermentation	= Yellow
1 - Acid production	= Gas
2 - Lysine decarboxylation	= Purple
3 - Ornithine decarboxyl.	= Purple
4 - H_2S production	= Black
4 - Indole: Add Kovac's	= Red
5 - Adonitol fermentation	= Yellow
6 - Lactose fermentation	= Yellow
7 - Arabinose ferment.	= Yellow
8 - Sorbitol fermentation	= Yellow
9 - VP:	SKIP
10 - Dulcitol fermentation	= Yellow
10 - Phenylalanine deamin.	= Black
11 - Urease production	= Pink
12 - Citrate utilization	= Blue

^Follow the directions in the manual for inoculation of the chambers: Don't contaminate the inoculating needle or yourself! Don't forget to break off the inoculating needle and to remove the seal. Label the tube with the name of the m.o.

		Possible	Microorganism #1			Microorganism #2			Microorganism #3		
Chamber Test	+ Reaction	Score	Score	Num	Code	Score	Num	Code	Score	Num	Code
1 - Glucose fermentation	= Yellow	2		x	x		x	x		x	x

1 - Acid production	= Gas	1				x				x				x
2 - Lysine decarboxylation	= Purple	4			x	x			x	x			x	x
3 - Ornithine decarboxyl.	= Purple	2			x	x			x	x			x	x
4 - H_2S production	= Black	1				x				x				x
4 - Indole: Add Kovac's	= Red	4			x	x			x	x			x	x
5 - Adonitol fermentation	= Yellow	2			x	x			x	x			x	x
6 - Lactose fermentation	= Yellow	1				x				x				x
7 - Arabinose ferment.	= Yellow	4			x	x			x	x			x	x
8 - Sorbitol fermentation	= Yellow	2		x	x			x	x			x	x	
10 - Dulcitol fermentation	= Yellow	1				x				x				x
10 - Phenylalanine	= Black	4			x	x			x	x			x	x
11 - Urease production	= Pink	2			x	x			x	x			x	x
12 - Citrate utilization	= Blue	1				x				x				x
CODE					_ _ _ _ _				_ _ _ _ _				_ _ _ _ _	
IDENTIFICATION (Using code manual)					_____				_____				_____	

E. Discussion and Conclusions