

General Microbiology Laboratory

Lab Session 6 - Microbial Physiology

I: Selective & Differential Media, and Hydrolytic Enzymes

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

Introduction

The identification of microorganisms is based primarily on specific metabolic characteristics of organisms after they have been isolated. Selective and differential media are used to help us isolate and tentatively identify organisms. Staining techniques like the gram stain clarify morphology. 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 this laboratory (which generally takes two lab periods to complete) is to perform many of the most common isolation and biochemical identification procedures used in research, clinical, industrial and public health microbiology laboratories.

A. Review the following for reference:

1. Lab Manual: Exercises 12, 13, 15, 14, 15, 16, 17, 18.
Color Plates: V2, V4, V5, V6, VIII4, & X5.
2. Text: Pages 110-113 and 859-873.

B. Do the following during lab:

1. Work in 3 groups. Use the attached list as a guide for which microorganisms to inoculate into which media.
2. Use culture media as directed. Divide culture plates as needed for inoculations. Label the cultures well!
4. Inoculate media as directed (e.g. isolation of specific mo's via streak plates).
3. Incubate the cultures for 48 hours at 37 C.
5. BSL-2 mo's are noted with an asterisk*. Handle appropriately! See instructor for *Salmonella enteriditis*.

C. Techniques:

Follow the directions for the *type* of inoculation:

Isolated colonies

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

Smear

Use an inoculating loop to make a large inoculum

Spot

Use an inoculating loop to make a small inoculum

Stab

Use an inoculating needle to 'stab' the 'deep'

D. Inoculations and Results:

Culture Media	Form	Type	Inoc.	M.O.'s	Results/Reactions
Blood Agar	Plate	Differential	Isol.	<i>Streptococcus pyogenes</i> *	Alpha-Hemolysis = Greening, e.g. <i>Strep. pneumoniae</i> ; Beta-Hemolysis = Clearing, e.g. <i>Strep. pyogenes</i> ; Gamma-Hemolysis = No hemolysis, e.g. <i>Staph. epidermidis</i>
			Col.	<i>Staphylococcus epidermidis</i>	
				<i>Streptococcus salivarius</i>	
				<i>Staphylococcus aureus</i> *	
				<i>Neisseria sicca</i> *	
				<i>Candida albicans</i>	
Columbia Agar	Plate	Selective	Smr.	<i>S. epidermidis</i>	Colistin and Naladixic acid select for gram (+) mo's
				<i>S. aureus</i> *	
				<i>Proteus vulgaris</i> *	
				<i>E. coli</i>	
Mannitol Salt Agar	Plate	Sel & Diff	Smr.	<i>S. epidermidis</i>	7.5% NaCl is selective for <i>Staph.</i> sp. It is differential for <i>S. aureus</i> due to fermentation of mannitol (= Yellow)
				<i>S. aureus</i> *	
				<i>Enterobacter aerogenes</i>	
				<i>E. coli</i>	
Eosin Methylene Blue Agar	Plate	Sel & Dif	Smr.	<i>E. coli</i>	Dyes select for gram (-) mo's. <i>E. aerogenes</i> = Pink, <i>E. coli</i> = Green (Lactose fermentation), <i>Pseudomonas</i> sp = Clear.
				<i>Pseudomonas aeruginosa</i> *	
				<i>E. aerogenes</i>	
				<i>S. epidermidis</i>	

Culture Media	Form	Type	Inoc.	M.O.'s	Results/Reactions
MacConkey Agar	Plate	Sel & Dif	Smr	<i>E. coli</i> <i>S. epidermidis</i> <i>E. aerogenes</i> <i>Citrobacter freundii</i>	<p>Selective for gram (-) mo's due to cv and bile. Differential for lactose fermentors = Pink. Note: <i>E. coli</i> = ppt bile & pink</p>
Hektoen Agar	Plate	Sel & Dif	Smr	<i>E. coli</i> <i>Serratia marcescens</i> <i>S. enteriditis</i> * <i>P. vulgaris</i> * <i>E. aerogenes</i> <i>S. epidermidis</i>	<p>Selective for enteric pathogens. <i>Shigella</i> sp. = Green <i>Salmonella</i>. sp. & <i>Proteus</i> sp. = Blue-green & black <i>Enterobacter</i> sp. & <i>E. coli</i> = Salmon pink to orange.</p>
XLD Agar	Plate	Sel & Dif	Smr	<i>E. coli</i> <i>P. vulgaris</i> * <i>S. enteriditis</i> * <i>S. marcescens</i> <i>E. aerogenes</i> <i>S. epidermidis</i>	<p>Selective for enteric pathogens. <i>Shigella</i> sp. = Red. Lactose fermentors (e.g. <i>E. coli</i> = Yellow (& ppt bile)). <i>Proteus</i> sp. = Yellow & black centers. <i>Salmonella</i> sp. = Red & black centers</p>

Culture Media	Form	Type	Inoc.	M.O.'s	Results/Reactions
Tellurite Glycine Agar	Plate	Sel & Dif	Smr	<i>E. coli</i> <i>S. epidermidis</i> <i>S. aureus</i> * <i>Corynebacterium xerosis</i>	Selective for coagulase (+) staphylococci. Black = Coagulase production. Gray = Non-pathogenic <i>Staphylococcus</i> sp. and <i>Corynebacterium</i> sp.
Potato Dextrose Agar	Plate	Selective	Smr	<i>C. albicans</i> <i>S. epidermidis</i> <i>E. coli</i> <i>Saccharomyces. cerevisiae</i>	This is a selective medium for the isolation of fungi.
Mycobiotic Agar	Plate	Selective	Smr	<i>C. albicans</i> <i>S. epidermidis</i> <i>E. coli</i> <i>S. cerevisiae</i>	This is a selective medium for the isolation of pathogenic fungi. It contains the antibiotics cycloheximide and chloramphenicol.
Pseudomonas Agar	Plate	Sel & Dif	Smr	<i>Ps. aeruginosa</i> * <i>Ps. fluorescens</i> <i>E. coli</i> <i>S. epidermidis</i>	This medium is selective for Pseudomonas species because it contains a proprietary broad spectrum antimicrobial (Irgasan) that has no effect on pseudomonads. It is differential because glycerol added to the medium enhances pigment formation.

Culture Media	Form	Type	Inoc.	M.O.'s	Results/Reactions
Starch Agar	Plate	Hydrolysis	Spot	<i>Bacillus subtilis</i> ^^ <i>E. coli</i> <i>S. epidermidis</i> <i>Bacillus cereus</i> ^^	Tests for production of an extracellular enzyme that degrades polysaccharides. + Test: Add iodine and look for clearing. ^^ Put this mo on a plate by itself.
Skim Milk Agar	Plate	Hydrolysis	Spot	<i>B. subtilis</i> ^^ <i>E. coli</i> <i>S. epidermidis</i> <i>B. cereus</i> ^^	Tests for production of an extracellular enzyme that degrades casein. + Test: Clearing of media. ^^ Put this mo on a plate by itself.
Gelatin Deep	Tube	Hydrolysis	Stab	<i>B. subtilis</i> <i>E. coli</i> <i>P. vulgaris</i> * <i>B. cereus</i>	Tests for production of an extracellular enzyme that degrades gelatin. + Test: Media remains liquid at 4 C.
Spirit Blue Agar	Plate	Hydrolysis	Spot	<i>S. aureus</i> * <i>P. vulgaris</i> * <i>E. coli</i> <i>S. epidermidis</i>	Tests for production of an extracellular enzyme that degrades lipids. + Test: Clearing of media.
DNase Agar	Plate	Hydrolysis	Spot	<i>S. aureus</i> * <i>P. vulgaris</i> * <i>S. epidermidis</i> <i>S. pyogenes</i> *	Production of an extracellular enzyme that degrades DNA. + Test: Clearing of media

Culture Media	Form	Type	Inoc.	M.O.'s	Results/Reactions
Oxidase	Plate	Redox	Spot	<i>N. sicca</i> <i>E. coli</i> <i>P. aeruginosa</i> * <i>S. epidermidis</i> <i>S. pyogenes</i> * <i>S. salivarius</i>	<p>Tests for production of oxidase. Add oxidase disc to growth on BHIA plate. + Test for oxidase: Disc turns purple.</p>
Catalase	Plate	Redox	Spot	<i>Enterococcus faecalis</i> * <i>S. aureus</i> * <i>S. epidermidis</i> <i>N. sicca</i> <i>S. pyogenes</i> * <i>P. aeruginosa</i> *	<p>Tests for production of catalase. Add H₂O₂ to growth on BHIA plate. + Test for catalase: Bubbles</p>
Methyl Red	Broth	Redox	Loop	<i>E. coli</i> <i>E. aerogenes</i>	<p>Tests for mixed acid fermentation. Add several drops of methyl red to tube. + Test for acid production: Red color develops at interface.</p>
V-P	Broth	Redox	Loop	<i>E. coli</i> <i>E. aerogenes</i>	<p>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.</p>

Carbohydrate Fermentation Broths

Glucose Sucrose Mannitol Maltose Lactose

Broth*	Fermentation	Loop	<i>C. freundii</i>
			<i>E. aerogenes</i>
			<i>E. coli</i>
			<i>Alkaligenes faecalis</i>
			<i>P. vulgaris</i> *
			<i>S. marcescens</i>

Interpretation of Carbohydrate Fermentation:

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 8.4, it changes from red to yellow between pH 8.4 and 6.8, and it is yellow below pH 6.8.

Triple Sugar Iron Agar

Slant Butt Gas H₂S

Slant	Fermentation	Stab	<i>E. aerogenes</i>
		Swab	<i>C. freundii</i>
			<i>P. vulgaris</i> *
			<i>E. coli</i>
			<i>S. enteritidis</i> *
			<i>A. faecalis</i>

Yellow butt/red slant = glucose fermentation = YB/RS
 Yellow butt & slant = lactose/sucrose fermentation = YB/YS
 Splitting of agar = gas production = G
 Blackening = H₂S production = HS

Enterotube*	Multiple tests	Inject	Gram negative, Oxidase negative, Glucose positive, Rods. Examples: <i>E. coli</i> <i>E. aerogenes</i> <i>P. vulgaris*</i> <i>S. marcescens</i> <i>C. freundii</i> <i>S. enteriditis*</i>	<u>Chamber # - Test for...</u>	<u>+ Test Result</u>
				1 - Glucose fermentation	= Yellow
				1 - Acid production	= Gas
				2 - Lysine decarboxylation	= Purple
				3 - Ornithine decarboxyl.	= Purple
				4 - H ₂ S 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.

Chamber Test	+ Reaction	Possible Score	Microorganism #1		Microorganism #2				
			Score	Num	Code	Score	Num	Code	
1 - Glucose fermentation	= Yellow	2		x	x		x	x	
1 - Acid production	= Gas	1			x			x	
2 - Lysine decarboxylation	= Purple	4		x	x		x	x	
3 - Ornithine decarboxyl.	= Purple	2		x	x		x	x	
4 - H ₂ S production	= Black	1			x			x	
4 - Indole: Add Kovac's	= Red	4		x	x		x	x	
5 - Adonitol fermentation	= Yellow	2		x	x		x	x	
6 - Lactose fermentation	= Yellow	1				x			x
7 - Arabinose ferment.	= Yellow	4		x	x		x	x	
8 - Sorbitol fermentation	= Yellow	2		x	x		x	x	
10 - Dulcitol fermentation	= Yellow	1			x			x	
10 - Phenylalanine	= Black	4		x	x		x	x	
11 - Urease production	= Pink	2		x	x		x	x	
12 - Citrate utilization	= Blue	1							

IDENTIFICATION (Using code manual) _____

E. Discussion and Conclusions