

General Microbiology Laboratory

Lab 7 – Special Project 1: Isolation and Identification of Normal Flora

Introduction

The respiratory and gastrointestinal tracts are composed of a diverse and complex normal flora. The purpose of this project is to use the techniques and skills you have developed over the semester to isolate and identify several microorganisms from these body compartments. You will also attempt to isolate specific microorganisms from the nasopharynx (*S. aureus*) and mucous membrane (*C. albicans*) using highly selective media.

A. Review the following for reference:

1. Lab Manual: Exercise # 46 - Pages 281 to 283. Exercise # 48 - Pages 295 to 297.
2. Text: Chap 30 - Pgs 734 to 740 and Fig 30.17. Chap 35 – Pgs 859-871, Box 35.1, Fig 35.5 and Tabs 35.1 & 35.2.
3. Cautions: Handle all materials as if they were pathogenic, BSL-2 microorganisms. Label all cultures well.

B. Do the following during lab:

Project 1. Isolation and identification of microorganisms from your throat.

- Do a throat swab on yourself using a sterile swab.
- Inoculate a small portion of a blood agar (BA) plate. Dispose of the swab in the discard container.
- Do a streak plate (on the BA plate) to get isolated colonies. Incubate 24-48 hours at 37 C.
- Use a new BA plate to reisolate 3-4 morphologically different colonies.
- Repeat the above step if necessary to get well isolated colonies you can work with.
- Gram stain all isolates. Describe colony and microscopic morphology of isolates.
- Choose 3 to 4 that are different and transfer the isolates to brain heart infusion agar slants.
- Characterize the isolates: Gram stain, colony and microscopic morphology, metabolic reactions.
- Identify at least 2 different microorganisms, as close as possible, using the identification key provided.

Project 2. Isolation and identification of a gram negative rod from your gi tract.

- Collect a very small fecal sample using a sterile swab.
- Carefully transfer the fecal matter to a tube of sterile water.
- Dispose of the swab in the discard container.
- Mix the water/fecal sample very well.
- Transfer a loopful of the material to a plate of EMB agar and a plate of T-Soy Agar.
- Streak for isolated colonies. Incubate for 24 hours at 37 C.
- Transfer 2 isolated colonies to T-soy agar slants. Choose 2 different mo's.
- Characterize the isolates: Gram stain, colony and microscopic morphology, metabolic reactions
- Identify, as closely as possible, using the identification key provided.

Note: If available, you may identify the mo using an Enterotube.

Note: You can eliminate *E. coli* by choosing one mo that is lactose negative.

Project 3: Isolation of coagulase positive *Staphylococcus aureus* from your nose.

- Do a nasal swab and streak for isolated colonies on a plate of Tellurite Glycine Agar.
Incubate for 48+ hours at 37 C.
- Transfer a large, dark, shiny black colony (of gram positive cocci) to a tube of Trypticase Soy Broth.
Incubate for 24 hours at 37 C. Note: You must gram stain the isolate.
- Add two drops of the 24 hour culture to a tube of citrated rabbit plasma.
Incubate in a 37 C water bath checking every 30 minutes (up to 4 hours) for coagulation of the plasma. Incubate for 24 hours before calling the reaction negative.

Project 4: Isolation of yeasts from a mucous membrane.

- Do a mucous membrane swab and streak for isolated colonies on Mycobiotic Agar.
Incubate for 48 hours at 37 C.
- Gram stain an isolate and transfer a yeast colony to a tube of Brain Heart Infusion Broth and incubate.
- Add an inoculum of the isolated yeast to a tube of rabbit plasma and incubate for 2-3 hours at 37 C.
Place a drop of the suspension on a microscope slide and observe for development of germ tubes.
Germ tubes are appendages that are one half the width and >3 to 4 times the length of the cell.

Note:

- Project 1 and 2 will be done entirely at your own pace.
- Portions of Project 3 and 4 will be done as a class project at specific times.

C. Techniques:

You will utilize techniques that you have already mastered as a part of this lab course.

The following is a logical sequence for the isolation, characterization and identification of some common bacteria (Projects 1 and 2).

1. Isolation

a. Streak for isolated colonies and incubate:

For throat swabs or fecal samples use Blood Agar (BA), Brain Heart Infusion Agar (BHIA), or a selective media like EMB and incubate at 37 C for 24-48 hours. Incubation in a candle jar (i.e. 5% CO₂), or at lower temperatures (e.g. 32 C) will enhance the growth of specific mo's.

b. Re-isolate, if necessary, and maintain desired colony types:

Transfer to BA or BHIA plates, streak for isolated colonies and incubate as in #1 above. You should repeat this if you do not have well isolated colonies. When colonies are well isolated, transfer to a BA or BHIA slant, incubate and save in refrigerator (i.e. 4 C).

c. Streak for isolated colonies on highly selective media as necessary to isolate pathogenic fungi or bacteria. Reisolation is not necessary, but it is necessary to gram stain isolates.

2. Characterization

a. Gram stain isolated colonies.

b. For Gram Positive mo's, note especially the following items:

Microscopic morphology

Spore formation

Catalase production

Glucose fermentation

c. For Gram Negative mo's, note especially the following items:

Microscopic morphology

Pigment production

Oxidase production

Glucose fermentation

Lactose fermentation

3. Identification

1) Use the attached brief key to help identify your unknown microorganism.

Note: Follow the key in a step-wise fashion: answer question I vs II, then A vs B, then 1 vs 2, etc

Note: The key is very abbreviated and many genera and species of mo's are not included.

2) Do additional biochemical tests, as necessary and available, to help in your identification.

D. Results:

1. You will write up a report on your all four Projects in your notebook.

2. You will turn in the following materials for Projects 1 and 2:

-All BA or BHIA plates, all slants, and all stained microscope slides.

3. You will **not** turn in the swabs, or any of the biochemical test media for Projects 1 and 2.

4. You will **not** turn in any of the materials used in Projects 3 and 4.

- I. Cells are coccus in shape
- A. Gram positive, oxidase negative
1. Catalase produced
 - a. Glucose not fermented, stained cells in tetrads, coagulase (-)
 1. Yellow pigmented colonies, hydrolyzes lipids
 2. Red pigmented colonies, no lipid hydrolysis
 - b. Glucose fermented, stained cells generally in clusters, nitrate reduced
 1. Acid from mannitol, coagulase (+), Beta-hemolytic
 2. No acid from mannitol, no hemolysis on BA
 - a. Acid from fructose
 - b. No acid from fructose
 - c. Glucose fermented, stained cells in tetrads, nitrate not reduced
 2. Catalase not produced
 - a. Growth in 6.5 % NaCl
 - b. No growth in 6.5% NaCl
 1. Alpha-hemolysis
 2. Beta-hemolysis
 3. Gamma-hemolysis
- B. Gram negative, oxidase positive
1. Acid from glucose, colonies not pigmented
 2. Acid from glucose, pigmented colonies
 3. Glucose not fermented, diplococci, pigmented colonies
 4. Glucose not fermented, cells arranged in clusters, colonies not pigmented
- II. Cells are rod or cocco-bacillary in shape
- A. Gram positive
1. Endospores present in stained smears, catalase (+), aerobic, gelatin (+)
 - a. Aerobic, catalase (+), gelatin (+)
 - b. Strict anaerobe, catalase (-)
 2. No endospores present
 - a. Aerobic, catalase (+), gelatin (-)
 - b. Aerobic, catalase (+), acid-fast (+), very slow growth
 - c. Strict anaerobe
 - d. Microaerophilic, catalase (-), (easily con. w/ Strep sp)
- B. Gram negative, aerobic or facultatively anaerobic
1. Glucose not fermented, oxidase positive
 - a. Glucose oxidized, gelatin hydrolyzed, Caseinase produced, diffusable pigments
 - b. Glucose not oxidized, white or yellow colonies
 2. Glucose fermented, oxidase negative: Use Enterotube, or:
 - a. Lactose fermented
 1. Citrate utilized
 - a. MR(-), VP(+)
 - b. MR(-), VP(+), Encapsulated
 - c. MR(+), VP(-)
 2. Citrate not utilized
 - b. Lactose not fermented
 1. Red pigment at 25 C
 2. No red pigment
 - a. Indole produced
 1. H₂S produced
 2. No H₂S
 - b. Indole not produced, mannitol not fermented
 - c. Indole not produced, H₂S(+), mannitol fermented
 - d. Citrate (-), H₂S (-), MR(+)

Micrococcus sp
Micrococcus luteus
Micrococcus roseus
 Staphylococcus sp
Staph. aureus

Staph. epidermidis
Staph. saprophyticus
 Gaffkya sp.

Enterococcus sp.
 Streptococcus sp
Strep. pneumonia
Strep. pyogenes
 Streptococcus sp.

Neisseria & Moraxella sp
Neisseria sicca, mucosa et al
Neisseria subflava
Neisseria flavescens
Moraxella catarhalis

Bacillus sp.
 Clostridium sp.

Corynebacterium sp.
 Mycobacterium sp.
 Propioibacterium sp, Others
 Lactobacillus sp.

Pseudomonas sp.
 Alkaligenes sp.

Enterobacter aerogenes
Klebsiella pneumoniae
Citrobacter freundii
Escherichia coli

Serratia marcescens

Proteus vulgaris
Morganella morganii
Proteus mirabilis
Salmonella sp.
Shigella sp.

E. Discussion