IDENTIFICATION OF UNKNOWN BACTERIA

It is virtually impossible to identify bacteria based on physical characteristics alone. This is due to the fact that there are only a few basic shapes and physical features commonly seen in the prokaryotic world. Instead, biochemical testing has been used to make bacterial identification down to the “species” level. These schemes are based on creating and matching biochemical profiles of the production of enzymes, acids and gases by isolated pure cultures of a given microorganism. Identification schemes and flow charts can be found in reference texts such as “Bergey’s Manual of Determinative Bacteriology” or “The Prokaryotes”.

Each group of students will receive a TSA slant or broth containing a pure culture of an unknown bacterium belonging to the Family Enterobacteriaceae. It is the responsibility of the group to maintain stock cultures of the organism provided. Working stock cultures will be used to inoculate the various biochemical test media over the next several weeks and should be fresh and free from contaminants. A reserve stock culture should be made and after incubation and comparison with the original slant, kept with the original slant in the refrigerator.

It is critically important that aseptic techniques are used during transfers and inoculations to prevent contamination of your cultures. If contamination is suspected, you will be able to fall back to your reserve stock. If you fail to maintain a reserve stock you will not be able to recover your organism if disaster strikes. The instructor will not provide a new culture for you to start with in the middle of the unknown exercises.

It is your responsibility to:
- keep your organisms alive and fresh to run tests
- check with us if you question purity of your organism or your test results
- appropriately select media to identifying unknowns
- ask for help
- keep your bacteria in pure culture
- check your results in a timely fashion (which may be CRITICAL for certain tests)
- ask for ID books when you cannot find them (the Bergey’s Manual chapter is in eCampus)
- put everything back where you found it
- ask for media and/or test materials
- come to lab prepared to run tests (your lab exercises, notebook, etc.)
- NOT write in the above ID books

OBJECTIVES:

1. Maintain a pure culture of the unknown organism provided
2. Determine physical characteristics of the organism provided
3. Inoculate various biochemical tests and be able to read and understand the significance of each test, whether positive or negative.
4. Use the information generated by testing, along with the given reference flow chart and identification texts, to deduce the Genus and Species designation of the unknown organism provided.
5. Hand in a report of the testing performed (Unknown identification sheet), a journal of how you arrived at the identification you indicated and a TSA plate containing the unknown organism streaked to demonstrate isolated colonies. Each student will hand in their own separate report even though you have performed the work together as a group.
Remember it is import to keep your own journal and not to plagiarize other students in the group.

MATERIALS NEEDED:

TSA plates
TSA slants
TSB broths
Clean glass slides
Gram stain reagents
Oxidase strips and reagent
Various biochemical media (distributed in sets during subsequent lab periods)

THE PROCEDURES:

SCHEMATIC OF IDENTIFICATION PROCEDURE

<table>
<thead>
<tr>
<th>1st period</th>
<th>2nd period</th>
<th>3rd period</th>
<th>4th period</th>
<th>5th period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain</td>
<td>oxidase test</td>
<td>Decarboxylase broths</td>
<td>Casein hydrolysis</td>
<td>optional tests</td>
</tr>
<tr>
<td>Streak plate</td>
<td>catalase test</td>
<td>Deaminase agar</td>
<td>Lipid hydrolysis</td>
<td></td>
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<tr>
<td>TSA slant/TSB</td>
<td>O-F glucose</td>
<td>Gelatin hydrolysis</td>
<td>starch hydrolysis</td>
<td></td>
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<tr>
<td>SIM</td>
<td>O2 tests</td>
<td>Carbohydrates</td>
<td></td>
<td></td>
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<tr>
<td>TTC motility</td>
<td>nitrate test</td>
<td>Urea hydrolysis</td>
<td></td>
<td></td>
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<tr>
<td>IMViC tests</td>
<td></td>
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1st Session
1. Inoculate a TSA slant using an inoculum from the original culture you have been given.
2. Inoculate a TSB broth from the original culture.
3. Streak a TSA plate for isolated colonies using the 3 section method, using the original agar slant culture. You will check for purity of the culture with this plate as well as use it to characterize the colony structure.
4. Run tests listed in schematic above (see individual exercises for specifics on each test).
5. Incubate all cultures at 25 c or 37 C as directed by your instructor.
6. Gram stain your unknown organism using the TSB broth culture. Note the shape, arrangement and Gram reaction of your organism.
7. Place the original stock slant labeled with your group names and instructor in the refrigerator. Inoculate a new working culture each week to have a fresh culture to inoculate new media.

2nd Session
1. Observe your new slants looking carefully for signs of contamination. Compare to the original slant noting the color (note pigmentation), texture, opacity and odor.
2. Describe the colony morphology displayed by isolated colonies observed on the streaked plate (refer back to the Colony Morphology experiment).
3. Observe the biochemical reactions and record the results on your unknown identification sheet. Make any additional notes in your journal.
4. Run tests listed in schematic above (see individual exercises for specifics on each test).
5. Incubate at temperature recommended by instructor.
3\textsuperscript{rd} Session
1. Observe the biochemical reactions and record the results on your unknown identification sheet. Make any additional notes in your journal.
2. Run tests listed in schematic above (see individual exercises for specifics on each test).
3. Incubate at temperature recommended by instructor.

4\textsuperscript{th} Session
1. Observe the biochemical reactions and record the results on your unknown identification sheet. Make any additional notes in your journal.
2. Run tests listed in schematic above (see individual exercises for specifics on each test).
3. Incubate at temperature recommended by instructor.

5\textsuperscript{th} Session
1. Observe the biochemical reactions and record the results on your unknown identification sheet. Make any additional notes in your journal.
2. Run tests listed in schematic above (see individual exercises for specifics on each test).
3. Incubate at temperature recommended by instructor.

6\textsuperscript{th} Session
1. Read and record final tests results according to each separate procedure in the laboratory manual. Review the resources available in the laboratory (Bergey’s Manual and other reference textbooks). If you are still unsure of the identity of your unknown, inoculate other tests as suggested in the reference texts for next class.

IT IS YOUR DECISION ABOUT WHAT OTHER TESTS SHOULD BE RUN FOR THE IDENTIFICATION OF YOUR BACTERIUM. This decision should be made with the identification tables in mind, identifying which tests might further identify your organism.

2. Streak a fresh TSA plate for isolated colonies. This plate will be handed in with the final identification report.

7\textsuperscript{th} Session
Hand in your 1) Unknown identification sheet (per table),
2) your journal (per table) and
3) the streaked plate of the unknown (1 per group)
MEDIA LIST for unknown bacterial identifications

Ask for the medium. Most of this media is available presently. If out, fresh media can be made upon request.

<table>
<thead>
<tr>
<th>Media</th>
<th>Media</th>
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</thead>
<tbody>
<tr>
<td>Gelatin deep</td>
<td>DNAse agar plate</td>
</tr>
<tr>
<td>MacConkey or EMB agar plate</td>
<td>EMB agar plate</td>
</tr>
<tr>
<td>Methylene blue lipid agar plate</td>
<td>Thioglycolate broth</td>
</tr>
<tr>
<td>MRVP broth</td>
<td>Litmus milk</td>
</tr>
<tr>
<td>Nitrate broth</td>
<td>Blood agar plate</td>
</tr>
<tr>
<td>Uni-OF glucose</td>
<td>Malonate (discs)</td>
</tr>
<tr>
<td>ONPG discs</td>
<td>Mannitol salt agar plate</td>
</tr>
<tr>
<td>pH media (3, 5, 10)</td>
<td>Starch agar</td>
</tr>
<tr>
<td>Phenylalanine deaminase agar slant</td>
<td>Skim milk agar plate</td>
</tr>
<tr>
<td>Decarboxylase (Moeller’s) broths:</td>
<td>SIM</td>
</tr>
<tr>
<td>arginine decarboxylase (ADC) broth</td>
<td>Simmon citrate agar slant</td>
</tr>
<tr>
<td>lysine decarboxylase (LDC) broth</td>
<td>TTC motility MRVP broth TSIA slant</td>
</tr>
<tr>
<td>ornithine decarboxylase (ODC) broth</td>
<td>Urea broth</td>
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</table>

**sugars:** ASK for a sugar ............ we may have additional ones not listed

<table>
<thead>
<tr>
<th>Sugar</th>
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</thead>
<tbody>
<tr>
<td>adonitol</td>
<td>maltose</td>
<td>xylose</td>
</tr>
<tr>
<td>arabinose</td>
<td>mannitol</td>
<td>cellibiose</td>
</tr>
<tr>
<td>dextrose/glucose</td>
<td>mannose</td>
<td>malonate</td>
</tr>
<tr>
<td>dulcitol</td>
<td>melibiose</td>
<td>lactose</td>
</tr>
<tr>
<td>esculin</td>
<td>raffinose</td>
<td>inulin</td>
</tr>
<tr>
<td>fructose/levulose</td>
<td>rhamnose</td>
<td>trehalose</td>
</tr>
<tr>
<td>galactose</td>
<td>salicin</td>
<td>inositol</td>
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<td></td>
<td>sorbitol</td>
<td>sucrose</td>
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</tbody>
</table>

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<table>
<thead>
<tr>
<th><strong>GRAM REACTION</strong></th>
<th>DNAse</th>
<th><strong>Casein hydrolysis</strong></th>
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</thead>
<tbody>
<tr>
<td><strong>MICROSCOPIC MORPHOLOGY AND ARRANGEMENT</strong></td>
<td><strong>Starch hydrolysis</strong></td>
<td><strong>Urea hydrolysis</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Gelatin hydrolysis</strong></td>
<td><strong>Lipid hydrolysis</strong></td>
</tr>
<tr>
<td></td>
<td><strong>ONPG</strong></td>
<td><strong>TSIA</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Nitrate reduction</strong></td>
<td><strong>DNAse</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>DNAse</strong></td>
</tr>
</tbody>
</table>

**DESCRIPTION OF COLONY:**

Color (clear, white, pigmented (if so, what color?)

Distinctive colony characteristics:
- colony shape/type of margin
- elevation of colony
- other?

**GROWTH CHARACTERISTICS:** *Circle one* for each

O2 needs: aerobe or facultative anaerobe?

Optimal growth T: 25°C  30°C  37°C

O-F glucose: oxidative, fermentative, or nonfermenter?

**BIOCHEMICAL TESTS (+ or -?):**

Oxidase

Catalase

Indole

Methyl red

Voges-Proskauer

Citrate

Motility

H₂S

Phenylalanine deaminase

Decarboxylase tests:
- Arginine
- Ornithine
- Lysine

**THE BACTERIUM WAS IDENTIFIED AS**

___________________  __________________
(genus)             (species)