Experiment 7	**Bacterial Phenotypes: a few identification Characteristics**
Advisor	NN
Reading	Chapters in BBOM 9th: 3.1; 21.2; 13.10 Chapters in BBOM 10th: 4.1; 24.2; 12.11 BBOM: Madigan M.T., J.M. Martinko and J. Parker: "Brock - Biology of Microorganisms", 9th Edition, 1999. 10th Edition, 2003, Prentice Hall. Chapters 1.2.3; 14.1 in White David: "The Physiology and Biochemistry of Prokaryotes", 2nd ed. Oxford, University Press, 2000. ISBN0-19-508439-X. Chapters 2.2; 11.3.2 in Lengeler J.W., G.Drews, H.G.Schlegel (eds): "Biology of the Prokaryotes", Thieme Stuttgart, 1999. ISBN: 3-13-108411-1. Chapters 6.3; 6.4; 2.2.4; in Schlegel H. G.: "Allgemeine Mikrobiologie", 7. Auflage, Thieme Stuttgart, 1992. ISBN: 3-13-444607-3.
Objectives	1. **Enrichment** of different bacteria originating from several sources 2. **Isolation** of **pure cultures** 3. **Characterization** of unknown organisms employing a few simple **diagnostic tests** for **phenotypic traits**
Background	In this exercise, you will enrich microorganisms from the following inocula: saliva, nose, feces and skin. Cultures will grow on blood agar and MacConkey agar. The cultures are characterized by **phenotype**: shape, spores (microscopy), Gram-staining, testing for catalase and oxidase-activity etc. We will distribute the work within the group. Each student will enrich one media using one of the inocula mentioned above and isolate **pure cultures**. In a second step, you will characterize one organism that you choose employing the diagnostic tests described below. **Gram staining:** based on differences in the **cell wall structure** (mainly the peptidoglycan layer) of Gram+ and Gram- bacteria (see BBOM 9th , pgs 51-52 and pgs. 68-77). **Catalase:** enzyme present in bacteria that need to destroy **toxic oxygen compounds** like H_2O_2. H_2O_2 is one of the byproducts which are produced by certain bacteria during the reduction of O_2 to H_2O in respiration (BBOM fig. 5.23). Catalase detoxifies H_2O_2 by oxidizing O with oxidation state I- to elemental O (oxidation state 0) and simultaneously reducing O(I-) to O(II-) as in H_2O according to the equation $H_2O_2 + H_2O_2 \rightarrow 2\ H_2O + O_2$ (BBOM 9th pgs 160-161) **Oxidase:** The **cytochrome oxidase** enzyme in aerobic bacteria catalyzes the transport of electrons from a donor compound to oxygen. In the oxidase test, the cytochrome oxidase is reduced. **Identification of *E. coli* with different biochemical tests:** **Adonite:** **Degradation of adonite** leads to a decrease in pH which is shown by the yellow color of the medium. **Malonate:** **Degradation of malonate** leads to an increase of pH which is shown by the blue color of the medium. **Gas from glucose:** Examination of **gas production from glucose**, if the medium turns yellow and gas is visible: positive test **Lysine-Decarboxylase:** detects the ability of an organism to **decarboxylate lysine**. The medium contains low amounts of glucose: first, medium turns yellow because of acid production from glucose degradation, second, lysine is decarboxylated (releasing CO_2) to cadaverin, the medium turns violet. **MIO (Motility/Indol/Ornithin):** The low agar concentration allows limited **movement of the motile bacteria**. Small amount of glucose and presence of L-

	Ornithine leads to an increase in pH (medium turns blue-violet). **DNase:** identifies bacteria capable of producing the **exoenzyme DNase**. The agar contains DNA and methyl green dye, which form a green complex. Bacteria which secrete DNase will hydrolyze the DNA. This results in clearing the agar around the bacterial colony. **TSI (Triple Sugar Iron Agar):** to differentiate bacteria based on their ability to **ferment glucose, lactose and/or sucrose**, and to **reduce sulfur to hydrogen sulfide**.
Literature	Kayser F. H., K. A. Bienz, J. Eckert, R. M. Zinkernagel: "Medizinische Mikrobiologie", 9. Auflage, Thieme Stuttgart, 1998. ISBN:3-13-444809-2
www. Links	Gram stain: http://health.upenn.edu/bugdrug/antibiotic_manual/gram.htm Biochemical tests: http://www2.austin.cc.tx.us/microbugz/labindex.html
Practical Work	1. Mix the tube containing your inoculum. Distribute the inoculum onto appropriate agar plates. Fractionate it on the plate using a plastic loop. 2. Incubate the plates for 24 hours at 37°C. 3. Examine the plates carefully without opening them (you will use plates of a group that did this experiment a day ago). Note the types of colonies; look especially for such features as pigmentation, texture, transparency, and shape. Pick out one well-separated colony for further examination. Mark it. Describe and draw gross appearance of the colony; place the plates on the microscope stage in an inverted position and use the low power objective. **Gram staining:** (perform the staining in a plastic dish and wear gloves) 1. Place a loopful of water onto a slide. Touch the colony with a loop and mix the bacteria with the water until there is a uniform, thin film of bacteria on the slide. "Air dry" the sample. 2. "Heat fix" the slide over a flame while gently moving the slide across the flame. Allow the slide to cool. 3. Stain with Crystal Violet for 1 minute by flooding the slide with stain. Rinse with water. 4. Apply Iodine solution for 1 minute by flooding the slide. Rinse with water. 5. Quickly (< 30 sec) decolorize the slide with decolorizer (ethanol/acetone-mixture, 50:50). Rinse with water. 6. Counterstain with saffranin solution for 1 min. Rinse with water. 7. Dry the slide by shaking and "air dry" the slide. 8. View organisms using the oil immersion objective and immersion oil (Gram-positiv cells are purple brown, Gram-negativ cells are pinkish-red) **Catalase test:** 1. Add a drop of hydrogen peroxide to a slide. 2. Add a loopful of the organisms to be tested and observe for immediate bubbling (Catalase positive organisms will exhibit bubbling) **Oxidase test:** 1. Place an adsorbent paper in a petri dish 2. Add a drop of oxidase-reagent (phenylendiamine) 3. Touch the colony with a loop and smear it on the oxidase-reagent 4. If oxidase positive the culture turns purple within 15 seconds (the test must be read within 15 seconds because the reagent is slowly oxidized by atmospheric oxygen) **Biochemical tests:** 1. Touch the *E. coli* colony once with a plastic loop and transfer the bacteria in the first, second, third test tube and so on. 2. Incubate for 18-24 h at 37°C 3. Analyze: color change, gas production, clouding of the medium (motility), clearing of the agar
Materials and	4 blood agar plates and 2 MacConkey agar plates, slides, adsorbent paper, petri

Experimental Protocols

dish, microscope, loops, gloves
Chemicals: Gram stain kit, hydrogen peroxide (20 ml), oxidase reagent, 1 biochemical set

Laboratory Rules & Precautions

General lab rules apply (wear lab coat, no eating, drinking, smoking, etc.)
It is necessary to work cautiously and aseptically. Use **good laboratory practice**! Do not contaminate yourself, others or the laboratory environment. All waste must be sterilized before disposal.
Please wash your hands before you leave the room and desinfect bench surfaces with 70 % ethanol.

Goals & Experiences gained

- Identification of the organisms that you have enriched for
- Learning about short diagnostic tests
- Examining different colonies under the microscope

Timing

90 minutes

Reporting

colony	Inoculum	Gram stain	color and shape		catalase	oxidase	
1							
2							
3							
4							
5							

Report in this table the test results of your colony (as colony 1). In addition, fill in the results of the other students in your group (colonies 2 to 5).

Results of the biochemical identification:
Malonate:
. . . . . .
Adonite:
. . . . . .
Gas from glucose:
. . . . . .
Lysine-Decarboxylase:
. . . . . .
MIO (Motility/Indol/Ornithin):
. . . . . .
DNase:
. . . . . .
TSI (Triple Sugar Iron Agar):
. . . . . .

Questions to be answered

What other diagnostic tests could be used for phenotype characterization?