Exercise 10.1 Identification of Bacterial Unknowns

LEARNING OUTCOMES

1. Apply deductive reasoning to determine appropriate tests for identification of bacteria.
2. Correctly identify two bacterial unknown isolates.

You will receive two bacterial unknown isolates from your instructor. Your task is to identify the genus and species of each unknown using the techniques and tests that you've learned throughout the semester. A dichotomous key and table of biochemical reactions is provided in this module to help. It is your responsibility to keep track of your unknown letters, reactions, and results throughout this project.

PERIOD 1

OBJECTIVE

This period is used to determine the Gram reaction and cellular morphology of each unknown, to prepare isolation streak plates for each unknown, and to perform preliminary testing.

MATERIALS

- EQUIPMENT: Inoculating loops, labeling tape, incinerator, sterile cotton applicators, glass slides, marking pen
- MEDIA: TSA plates, nitrate broth
- SOLUTIONS: Gram stain materials, 3% hydrogen peroxide, oxidase reagent
- CULTURES: Bacterial unknown and control

cultures PROCEDURE – STUDENTS WORK INDIVIDUALLY

1. Obtain two unknown cultures from the instructor and record the letters in your notes.
2. Streak each unknown for isolation onto a TSA plate and place it in a common rack for incubation at 37^{o}C for 18-24 hours.
3. Determine the Gram reaction, shape, and arrangement of cells for each unknown. When preparing slides, include additional slides with known Gram-positive and Gram-negative bacteria as controls. Examine stained slides microscopically under oil immersion and record results. You may wish to save heat-fixed or stained slides (blot excess oil) in a box.
4. Aseptically inoculate each unknown into a nitrate broth tube.
5. Time permitting, perform a catalase and oxidase test for each unknown.
6. When you are done, return unknown culture tubes to the instructor.

PERIOD 2

OBJECTIVE

This period is used to determine colony morphology and to inoculate secondary test media for each unknown.

MATERIALS

- EQUIPMENT: Inoculating loops, labeling tape, incinerator, marking pen, wooden sticks
- MEDIA: SIM deeps, MRVP broth, citrate slants, urease broth, coagulase tubes, bile esculin slants, agar plates (BAP, EMB, MAC, MSA, cetrimide)
- SOLUTIONS: Nitrate reagents (A, B, zinc powder)
- CULTURES: Bacterial unknown subculture plates from Period 1; control cultures

PROCEDURE – STUDENTS WORK INDIVIDUALLY

1. Examine unknown subculture plates for isolated colonies and record colony color, shape, and margin for each culture.
2. Examine results of the nitrate reduction test, adding reagents where appropriate. Record results for each unknown culture.
3. Follow the dichotomous key to select appropriate secondary tests required to identify each unknown based on Gram reaction, nitrate, oxidase, and catalase results for each unknown.

NOTE: Setting up all tests for both unknowns is costly and unnecessary. Follow the dichotomous key to work deductively and inoculate only those tests which apply.

4. Use isolated colonies from each subculture plate to inoculate the appropriate secondary media. Your instructor may assign a common set of control bacteria for biochemical tests to be set up by different students rather than having each student set up controls individually.
5. Place secondary test tubes and media in a common rack for incubation at 37^{o}C for 18-24 hours. If your unknown requires incubation at 25^{o}C, let the instructor know.
6. When you are done, remove tape from the nitrate broth tubes and place them in a common rack for autoclaving; return the subculture plates for both unknowns to the instructor.

PERIOD 3

OBJECTIVE

This period is used for follow-up secondary testing on each unknown and for completing the final report.

MATERIALS

- EQUIPMENT: Disposable Pasteur pipets and small glass serological tubes, wooden stick
- SOLUTIONS: Nitrate reagents, MRVP reagents, zinc dust, UV lamp
- CULTURES: Bacterial unknown subculture plates from Period

1 PROCEDURE – STUDENTS WORK INDIVIDUALLY

1. Perform secondary tests for each unknown, adding reagents where necessary. Record results.
2. When you are done, remove tape from all tubes and place them in a common rack for autoclaving; dispose of plates in the Petri plate discard bucket. Small serological tubes for MRVP and coagulase tests should be disposed of in the disinfectant beaker.
3. Complete the final report, indicating the identity of each unknown and the test results on which the conclusion was based.