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# *Laboratory Experiments in Microbiology*

*Ted R. Johnson  Christine L. Case*

*Tenth Edition*

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## Laboratory Experiments in Microbiology

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# Unknown Identification and *Bergey's Manual*

*The strategy of* **DISCOVERY** *lies in determining the sequence of choice of problems to solve. Now it is in fact very much more difficult to see a problem than to find a solution to it. The former requires* **IMAGINATION**, *the latter only ingenuity.* – **JOHN BERNAL**

## Objectives

After completing this exercise, you should be able to:

1. Explain how bacteria are characterized and classified.
2. Use *Bergey's Manual*.
3. Identify an unknown bacterium.

## BACKGROUND

In microbiology, a system of classification must be available to allow the microbiologist to categorize and classify organisms. Communication among scientists would be very limited if no universal system of classification existed. Classification of microorganisms is based on similarities in their ribosomal RNA (rRNA). Bacteria and archaea with similar rRNA sequences are grouped together into taxa. The taxa used for prokaryotes are domain, phylum, class, order, family, genus, and species. Bacteria are *identified* for practical purposes—for example, to determine an appropriate treatment for an infection. They are not necessarily identified by the same techniques by which they are *classified*. Most identification procedures are easily performed in a laboratory and use as few procedures or tests as possible.

The most important reference for bacterial taxonomy is ***Bergey's Manual.**** *Bergey's Manual of Systematic Bacteriology* provides classification based on rRNA. *Bergey's Manual of Determinative Bacteriology* is a valuable reference in the lab because it provides identification (determinative) schemes based on such criteria as morphology, differential staining, oxygen requirements, and biochemical testing. Different tests are required, depending on the bacterium being identified. A test that is critical for distinguishing one bacterial group from another may be irrelevant for identifying other bacteria. The criteria listed in *Bergey's Manual* and used to develop the keys in Appendix: Keys to Bacteria are relatively constant, although atypical bacteria will be found through repeated laboratory culture. This variability, however, only heightens the fun of classifying bacteria.

You will be given an unknown heterotrophic bacterium to characterize and identify. By using careful deduction and systematically compiling and analyzing data, you should be able to identify the bacterium.

Keys to species of bacteria are provided in Appendix: Keys to Bacteria (see Figures 1 and 2 in Appendix: Keys to Bacteria). These keys are an example of a dichotomous classification system—that is, a population is repeatedly divided into two parts until a description identifies a single member. Such a key is sometimes called an "artificial key" because there is no single correct way to write one. You may want to check your conclusion with the species description given in *Bergey's Manual*.

To begin your identification, ascertain the purity of the culture you have been given, and prepare stock and working cultures. Avoid contaminating your unknown. Note growth characteristics and Gram-stain appearance for clues about how to proceed. After culturing and staining the unknown, you will be able to eliminate many bacterial groups. Final determination of your unknown will depend on your carefully selecting the relevant biochemical tests and weighing the value of one test over another in case of contradictions. Enjoy!

## Materials

Petri plates containing trypticase soy agar (2)
Trypticase soy agar slant (2)
All stains, reagents, and media previously used

## Cultures

Unknown bacterium # _____

## Techniques Required

Compound light microscopy
Hanging-drop procedure
Wet-mount technique

**Bergey's Manual of Systematic Bacteriology,* 2nd ed., (2004), is the reference for classification. *Bergey's Manual of Determinative Bacteriology,* 9th ed. (1994), is used for laboratory identification of culturable bacteria and archaea.

Inoculating loop and needle technique
Aseptic technique
Negative staining
Gram staining
Acid-fast staining
Endospore, capsule, and flagella staining
Plate streaking
OF test
Starch hydrolysis
MRVP tests
Fermentation tests
Citrate test
Protein catabolism
Catalase test
Nitrate reduction test
Oxidase test

## PROCEDURE First Period

1. Streak your unknown onto the trypticase soy agar plates for isolation. Incubate one plate at 35°C and the other at room temperature for 24 to 48 hours. Note the growth characteristics and the temperature at which each one grows best.
2. To help you begin, try to identify the bacteria in FIGURE 1 using Appendix: Keys to Bacteria. The results of one test, such as the Gram stain, help you plan the next test. (The unknown in Figure 1 is a gram-positive coccus. According to Figure 2 in Appendix: Keys to Bacteria, a catalase test should be done next.)

## PROCEDURE Second Period

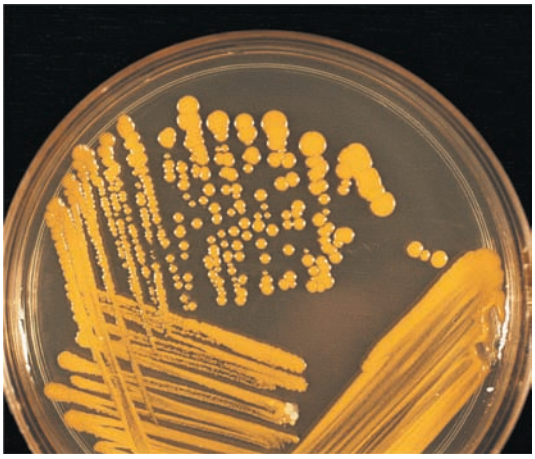
1. Aseptically inoculate two trypticase soy agar slants from a colony on your streak plate. Incubate them for 24 hours. Describe the resulting growth. Keep both slant cultures in the refrigerator. Designate one as your stock culture; the other as your working culture. Subculture your stock culture onto another slant when your working culture is contaminated or not viable. Keep the working culture in the refrigerator when it is not in use.

## PROCEDURE Next Periods

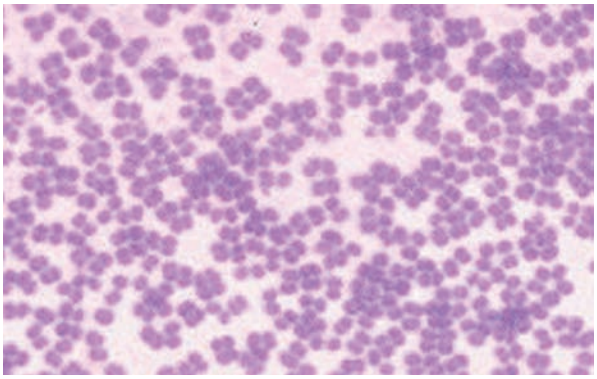
1. Use your working culture for all identification procedures. When a new slant is made and its purity demonstrated, discard the old working culture. What should you do if you think your culture is contaminated? _____

   _____
2. Read the keys in Appendix: Keys to Bacteria to develop ideas on how to proceed. Perhaps determining staining characteristics might be a good place to start. What shape is it? _____ What can be eliminated? _____

   Not all bacteria are included in Appendix: Keys to Bacteria. You should check *Bergey's Manual of Determinative Bacteriology.*
3. After determining its staining and morphologic characteristics, determine which biochemical tests you will need. Do not be wasteful. Inoculate *only* what is needed. It is not necessary to repeat a test—do it once accurately. Do not perform unnecessary tests.
4. If you come across a new test—one not previously done in this course—determine whether it is essential. Can you circumvent it? _____ If not, consult your instructor.
5. Record your results in the Laboratory Report, and identify your unknown.

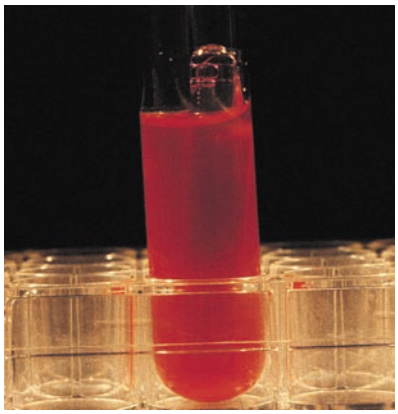


**(a) The unknown organism is isolated in pure culture**.

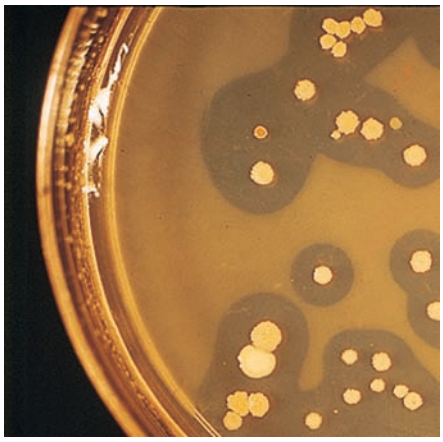


**(b) A Gram stain is made from the pure culture**.

**(c) Based on the result of the Gram stain, a catalase test is indicated**.



**(d) Results of the previous tests lead to the next series of tests**. This photograph shows the results of a glucose fermentation tube inoculated with the unknown bacterium.



**(e) Lipid hydrolysis test**. The clearing around the colonies indicates hydrolysis of the lipid in the medium.

**FIGURE 1 Unknown Identification.** This is an example of how to identify an unknown bacterium. Use these test results and Appendix: Keys to Bacteria to identify the bacterial species shown in the photographs. CDC

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Name: _____ Date: _____ Lab Section: _____

LABORATORY REPORT

# Unknown Identification and *Bergey's Manual*

## PURPOSE

_____

_____

_____

## RESULTS

Unknown # _____

| Morphological, Staining, and Cultural Characteristics | |
|---|---|
| The cell<br>Staining characteristics<br>Gram _____ Age _____<br>Other _____ Age _____<br>Shape _____<br>Size _____<br>Arrangement _____<br>Endospores (position) _____<br>_____<br>Motility _____<br>Determined by _____ | Colonies on trypticase soy agar<br>Diameter _____<br>Appearance _____<br>Color _____<br>Elevation _____<br>Margin _____<br>Consistency _____<br>Agar slant Age _____<br>Amount of growth _____<br>Pattern _____<br>Color _____ |

Make a table listing the tests you performed and their results. Remember to do only the necessary tests and to avoid repeating a test.

## CONCLUSIONS

1. What organism is shown in Figure 1? _____

2. What organism was in unknown # _____ ? _____

## QUESTIONS

1. On a separate sheet of paper, write your rationale for arriving at your conclusion.

2. Why is it necessary to complete the identification of a bacterium based on its physiology rather than its morphology? _____