

Objective:

The purpose of this laboratory exercise is to identify an “unknown” bacterium using the Biolog GEN III Microbial Identification System. The GEN III system is used to analyze the ability of the cell to metabolize all major classes of biochemicals, in addition to determining other important physiological properties such as pH, salt, and lactic acid tolerance, reducing power, and chemical sensitivity.

Historical Background:

Dutch scientist L.E. Den Dooren de Jong proved that microbes could be distinguished based on their utilization of different sets of carbon sources and based his doctoral thesis on this discovery in 1926. How the individual cells responded to different carbon sources was the foundation used for the next 80 years to bring us to today’s view of bacterial taxonomy. All of the original work in bacterial taxonomy was based on **phenotypic** properties.

phe·no·type () n. The observable physical or biochemical characteristics of an organism, as determined by both genetic makeup and environment.

Pheno derives from the Greek for *display*: the phenotype is the manifestation of the genetic make-up of the individual.

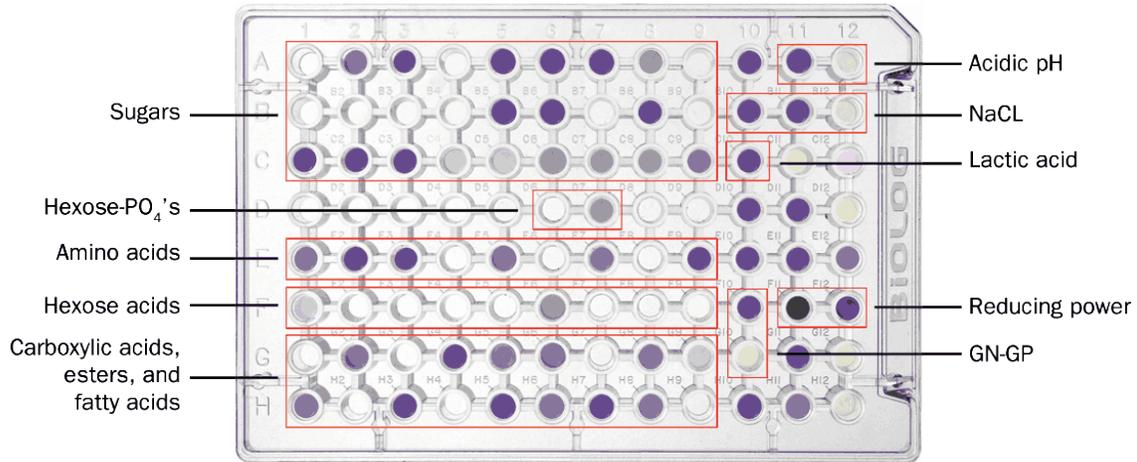
Method Overview:

The Biolog GEN III MicroPlate analyzes a microorganism in 94 phenotypic tests: 71 carbon source utilization assays and 23 chemical sensitivity assays. The test panel provides a “Phenotypic Fingerprint” of the microorganism that can be used to identify it to the species level. All necessary nutrients and biochemical are prefilled and dried into the 96 wells of the MicroPlate. Tetrazolium redox dyes are used to colorimetrically indicate utilization of the carbon sources or resistance to inhibitory chemicals.

The isolate to be identified is grown on agar medium and then suspended in a special “gelling” inoculating fluid (IF) at the recommended cell density. Then the cell suspension is inoculated into the GEN III MicroPlate, 100 µl per well, and the MicroPlate is incubated to allow the phenotypic fingerprint to form. All of the wells start out colorless when inoculated. During incubation there is increased respiration in the wells where cells can utilize a carbon source and/or grow. Increased respiration causes reduction of the tetrazolium redox dye, forming a purple color. Negative wells remain colorless, as does the negative control well (A-1) with no carbon source. There is also a positive control well (A-10) used as a reference for the chemical sensitivity assays in columns 10-12. After incubation, the phenotypic fingerprint of purple wells is compared to Biolog’s extensive species library. If a match is found, a species level identification of the isolate is made.

Anatomy of a GEN III ID:

ID = *Stenotrophomonas maltophilia*



71 Carbon Sources plus 23 Chemical Sensitivity Assays

Safety:

Dispose of Biohazards properly - including used culture plates, Inoculatorz, Inoculating Fluid, pipette tips and reservoirs. Use caution when working with live cultures and follow all procedures and guidelines of your facility. Use of gloves, lab coats and safety glasses is required.

Basic Techniques:

Before you begin make sure you are confident with:

1. Aseptic technique
2. Streaking a plate for isolated colonies
3. Using a multi-channel pipettor

Materials:

- GEN III MicroPlates: Catalog No. 1030 Biolog GEN III MicroPlates (10/box)
- Agar Culture Media: Catalog No. 71102 BUG Agar with 5% sheep blood (BUG+B)
- Inoculating Fluid A (IF-A): Catalog No. 72401
- Inoculatorz: Catalog No. 3321- Sterile disposable inoculator swabs (20x50) or Catalog No. 3323 (100x1 – individually wrapped).
- Reservoirs: Catalog No. 3102- Sterile disposable reservoirs.
- PipetTips: Catalog No. 3201 Sterile racked pipet tips for Ovation multichannel pipettor; or Catalog No. 3001- Matrix multichannel pipettor tips.

PROCEDURE:

DAY ONE: Culture Organism on Biolog Recommended Agar Media

Isolate a pure culture on Biolog recommended agar media (BUG+ B) and incubate at 33°C. The cells must be freshly grown since many strains lose viability and metabolic vigor in stationary phase. The recommended incubation period for most organisms is 4-24 hours. Spore-forming gram-positive bacteria (*Bacillus* and related genera) should be grown for less than 16 hours to help minimize sporulation.

DAY TWO: Prepare Inoculum and set up MicroPlate

Before starting, prewarm MicroPlates and IF-A to room temperature and review the entire protocol.

Blank the turbidimeter with a clean tube (wiped clean of dirt and fingerprints) containing uninoculated IF. Because the tubes used are not optically uniform, they should be blanked individually. Set the 100% transmittance adjustment knob so that the meter reads 100%.

Prepare the inoculum at the desired turbidity. The target cell density should be in the range of 90-98%T.

Use a cotton-tipped Inoculatorz swab to pick up a 3 mm diameter area of cell growth from the surface of the agar plate. Release the bacteria into the IF by rubbing the swab tip against the bottom of the tube containing IF. Crush any cell clumps against the tube wall or remove them from the IF by catching them on the swab. Stir the IF with the swab to obtain a uniform cell suspension and read it in the turbidimeter. If the cell density is too low, add more cells. If the cell density is too high, add more IF.

Pour the cell suspension into the multichannel pipet reservoir.

Fasten 8 sterile tips securely on to the 8-Channel Repeating Pipettor and fill the tips by drawing up the cell suspension from the reservoir.

Fill all wells with precisely 100 µl. Be careful not to carry over chemicals or splash from one well into another. The inoculating fluid will form a soft gel shortly after inoculation.

Cover the MicroPlate with its lid and eject the pipettor tips.

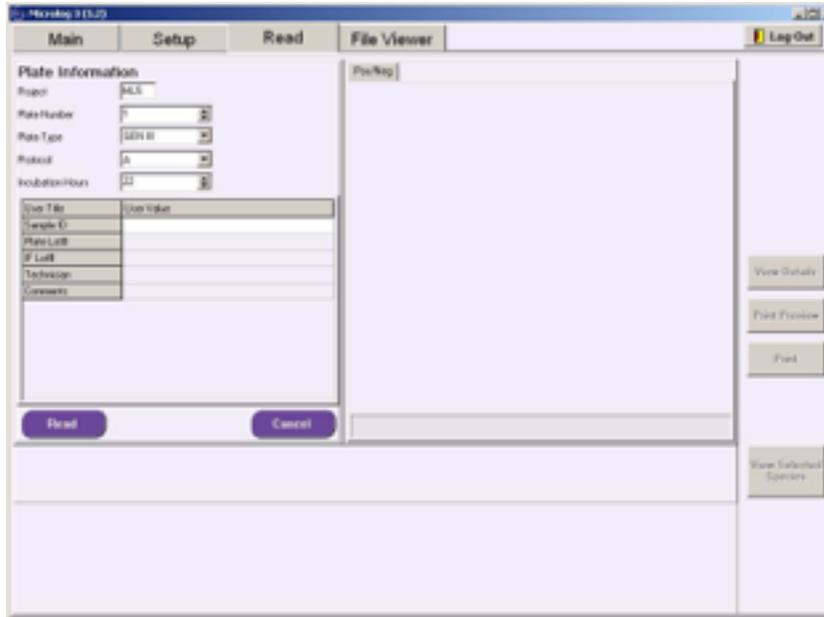
Incubate MicroPlate in an incubator set at 33° C for 3 to 36 hours.

DAY THREE: Reading and Interpretation of Results

MicroPlates can be read using Biolog's MicroLog[®] manual microbial ID system, the semi-automated MicroStation[™] or the fully automated OmniLog[®]. For this exercise we will be using the MicroStation to read the MicroPlates and make a species identification.

Your instructor will have logged into the MicroStation software, initialized the reader and selected the appropriate database.

An image of the software screen is shown below.



- 1.) From the Read tab, select the Read Setup tab.
- 2.) Select Reader from the Input Mode drop-down box.
- 3.) Select "No" from the Use Batch drop-down menu.
- 4.) Select "Yes" from the Automatic Print-Out drop-down menu to automatically print out your result. Otherwise, select No.
- 5.) Select Yes or No in the Save To Data File drop-down menu. If you choose Yes, the Data File Name field displays. Click the Folder icon to populate the Data File Name field. Enter a new name in the File Name text box or choose a previously created data file used within the same user session and click Save.

6.) Click the Read New Plate button. The Plate Information screen displays.

Enter sample identifiers for the following fields:

- a. Project: Enter three alphanumeric characters for the project name or number.
- b. Plate Number: Enter the MicroPlate number. (This is an optional user field entry for non-batch reading.)

c. Plate Type: Select the type of MicroPlate from the drop-down list. You are using a GEN III MicroPlate.

d. Protocol: Select the protocol from the drop-down list for GEN III MicroPlates. Select Protocol A.

e. Incubation Hours: Enter the hours that the MicroPlate has incubated.

7. Check your MicroPlate before loading by:

a. Making sure the reaction pattern is well developed.

b. Wiping the bottom of the MicroPlate to remove condensation and fingerprints.

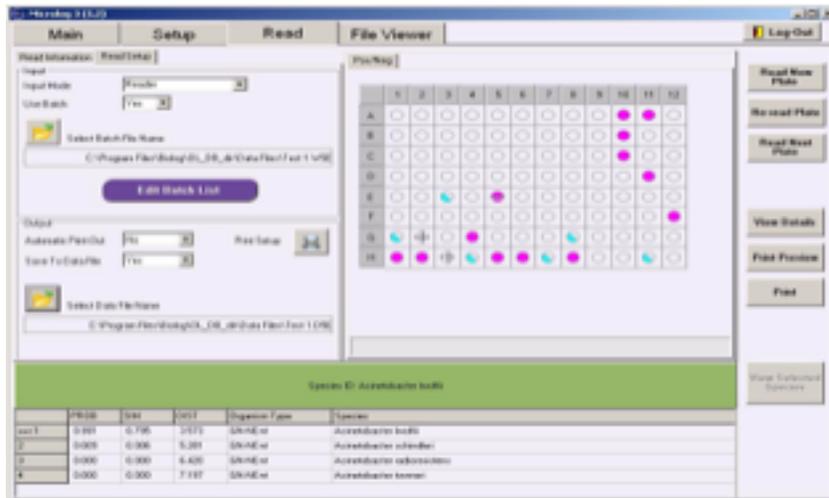
c. Placing the MicroPlate on the reader tray with the A1 well at the top left-hand corner.

8. Gently push the MicroPlate down until it snaps into a level position in the reader tray and remove the MicroPlate lid.

9. Click the **Read** button. The reader calibrates itself, pulls the MicroPlate inside, takes a reading, and displays the read results on the **Read Information** tab. The MicroPlate is ejected.

10. An example screen after the reading is shown below. The Final identification results are displayed in the ID Box (shown here in green with a species ID of *Acinetobacter Iwoffii*), located at the bottom of the Read screen. Entry #1 is the best match selected from the database.

If the answer to the following three questions is yes, you can feel confident that ID #1 is accurate.



- Are the top-ranked ID choices on the list all the same (or closely related) genera?
- Check the SIM (similarity index value) rating of ID #1. Is it greater than .5?
- Check the DIST (distance) rating of ID #1. Is it greater than two distance points away from the second choice.

Exercise Questions:

- 1.) Did you obtain a species level Identification? If not, can you provide any ideas on why an identification was not made?
- 2.) Did your identification result conform to the confidence criteria listed above?
- 3.) What is the significance of the A1 well?
- 4.) What is the significance of the A10 well?

Additional Links and Resources: ([need Yigal to link these](#))

[GEN III IFU \(Instructions for Use\)](#)

[GEN III Set-up Video](#)

[Biolog database species list](#)