

General Microbiology Laboratory

Lab Session 10 - Environmental Microbiology

I: The Effects of the Environment on Microorganisms, and II: The Effects of Microorganisms on the Environment

Introduction

Aquatic and terrestrial environments contain a large diversity of microorganisms, as well as a tremendous number of organisms. There are usually hundreds (even thousands) of species of bacteria in a gram of soil. There are typically a million organisms per gram of nutrient-poor soil, and there can be a billion or more organisms in nutrient-rich soil. These organisms all have specific growth and nutrient requirements, and so when one looks for organisms under only one set of conditions one sees only a limited diversity of organisms. These organisms not only sequester nutrients for their survival but they eliminate wastes in the process, and so they change their environment. There are two purposes to this lab:

- 1) To determine the range of bacteria typically present in a soil sample; and,
- 2) To determine the chemical changes that take place in soil as microorganisms metabolize.

A. Review the following for reference:

1. Lab Manual: Exercises 19, 20, 21 and 55.
2. Text: Chap 5, pgs 102-105; Chap 6, pgs 132-142; Chap 9, pgs 205-214; and, Chap 28, pgs 644-653.

B. Do the Following During Lab

1. Environmental Influences on Microbial Growth.

a. You will need the following materials for each soil sample:

- 1) Materials to prepare soil suspensions: 'Raw' soil sample, sterile tubes, balance, water, pipettes, etc
- 2) Materials to evaluate growth characteristics of bacteria:
 - 2 Trypticase soy agar plates: 1 each for aerobic and anaerobic culture
 - 7 Trypticase soy agar plates: 1 each for growth at 5, 15, 25, 35, 45, 55 & 65 C
 - 9 Trypticase soy broth tubes: 1 each for pH 2.5, 3.5, 4.5, 5.5, 7, 8.5, 9.5, 10.5 and 11.5.
 - 9 Trypticase soy broth tubes: 1 each for 0.5, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 & 20.0% NaCl.

b. You will do the following:

1) Prepare a soil suspension:

- Weigh 1 gram of soil and mix with 9 ml sterile water,
- Mix well and then allow the slurry to settle for several minutes,
- Use the upper portions of the mixture to inoculate culture media as directed.

2) Determine effects of environmental conditions on growth of bacteria:

Note: You will monitor growth on a semi-quantitative scale (- to +++) described below.

a) Effects of Oxygen

- Inoculate 2 TSA plates with 100 ul of the soil mixture, swab surface.
- Incubate 1 plate aerobically and 1 in the anaerobic jar, each at 25 C for 2-5 days.

b) Effects of Temperature

- Inoculate 7 trypticase soy agar plates with 100 ul of the soil mixture, swab surface.
- Incubate 1 plate at each of the following temps: 5, 15, 25, 35, 45, 55 & 65C for 2-5 days.

c) Effects of pH

- Inoculate 100 ul of soil sample into TSB at pH 2.5, 3.5, 4.5, 5.5, 7, 8.5, 9.5, 10.5 & 11.5.
- Incubate all at 25 C for 5-7 days.

d) Effects of Osmotic Pressure

- Inoculate 100ul soil sample into TSB tubes at 0.5, 2.5, 5, 7.5, 10, 12.5, 15, 17.5 & 20% NaCl.
- Incubate at 25 C for 5-7 days.

2. Microbial Effects on the Environment - The Nitrogen Cycle

a. You will need the following materials:

- Raw soil samples
- 4 tubes of Peptone Broth (for Part B.2.b.1 below) which contains a high concentration of proteins and peptides
- 4 tubes of Nitrate Broth with Durham tubes (for Part B.2.b.2 below) which contains peptides and glucose for carbon and energy and 4% nitrate salts as an electron acceptor.

-1 tube of Nitrite Broth and 1 tube of Ammonium Sulfate Broth (for Part B.2.b.3 below). These contain inorganic carbonates as sole carbon sources and reduced inorganic nitrogen (ammonium or nitrite) for energy.

b. You will do the following inoculations on Day 1:

1) Determination of Ammonification

(This is an assay for chemoheterotrophs)

- Inoculate 2 tubes of Peptone Broth with a pinch of raw soil sample and incubate.
- Incubate one tube for 2 days and one tube for 7 days, each at 25 C.
- Repeat using a control microorganism.

2) Determination of Denitrification

(This is an assay for anaerobic respirators)

- Inoculate 2 tubes of Nitrate Broth (with Durham tubes) with a pinch of raw soil.
- Incubate one for 2 days and one for 7 days, each at 25 C.
- Repeat using a control microorganism.

3) Determination of Nitrification

- Inoculate 1 tube of Nitrite Broth and 1 tube of Ammonium Sulfate broth with raw soil.
- Incubate both tubes for 7 days at 25 C.

4) Determination of Nitrogen Fixation

We will skip this assay.

C. Assays:

We are monitoring changes in the form of nitrogen as nitrogen is cycled by microorganisms in soil.

1) Ammonification: (Release of ammonia (NH_4^+) from organic sources via peptide hydrolysis and deamination)

- Add 2-3 drops of culture to 2-3 drops of Nessler's reagent on a spot plate.

Results: Pale yellow = minimal ammonia. Dark brown/ppt = lots of ammonia.

The intensity of the color change indicates the degree of mineralization in the culture.

2) Denitrification: (Reduction of nitrate to nitrite and even nitrogen gas (N_2) in anaerobic respiration)

- Add 5 drops culture to a spot plate and then 2 drops of Nitrate Reagent A and 2 drops of Nitrate Reagent B.

Results: Red (within 30 seconds) = Nitrite is present in culture.

- If the reaction does not turn red it indicates that either nitrate is still in the culture, or the nitrate was completely reduced to gaseous products (e.g. nitrogen gas). To distinguish these add a very small "pinch" of zinc powder. Zinc rapidly catalyzes the reduction of nitrate to nitrite.

Results: Red = Nitrate is present in culture. No color = No nitrate or nitrite is present.

Nitrogen gas may have been generated and may be seen in the Durham tube.

(The absence of any changes may also indicate assimilatory nitrate reduction.)

3) Nitrification: (Oxidation of ammonia to nitrite (NO_2^-) and then nitrate (NO_3^-) by lithotrophs)

The chemistry of these assays is very complex. Rather than attempt the assays, we simply monitor growth. The only organisms that can grow in the nitrite broth and the ammonium sulfate broth are chemoautolithotrophs. Carbon dioxide is the only source of carbon, and nitrite or ammonium is only oxidizable substrate as a source of energy.

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D. Results

1. Results: Environmental influences on growth.

a. Predict, in advance, under which conditions you will find growth or no growth, and why!!!

b. After incubation, observe each of the plates and tubes you inoculated for growth.

1) Prepare a table of results and use the following key as a qualitative estimate of growth:

-	No growth	+	Minimal growth
++	Moderate growth	+++	Heavy growth

2) Describe any unusual (fungal?) growth.

3) Was growth different from your prediction?

2. Results: Microbial effects on the environment.

a. Record results and discuss cycling of nitrogen.

Environmental Influences on the Growth of Microorganisms:

Oxygen	Aerobic	Anaerobic							
Temperature (C)	5	15	25	35	45	55	65		
pH	2.5	3.5	4.5	5.5	7	8.5	9.5	10.5	11.5
Osmotic Pressure (% NaCl)	0	2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0

Microorganisms Influences on the Environment: Nitrogen Cycling

Ammonification	Soil Sample	Control MO
Denitrification		
Nitrification		

E. Discussion