EXPERIMENT 6

FACTORS INFLUENCING THE GROWTH OF MICROBES

The survival and growth of a microbe is a function of a myriad of different activities: nutrient assimilation, catabolism, and the synthesis of living cytoplasm and all cellular structures. All these various activities are performed by specific enzymes. Each of these enzymes functions best when in the presence of the optimal environmental conditions of temperature, pH, osmotic pressure, and redox potential. The optimum conditions vary for each enzyme. In this experiment, four environmental parameters will be examined: temperature, oxygen, pH and salinity.

Temperature. Depending upon their optimum temperature for growth, bacteria are divided into three groups: **psychrophiles**, **mesophiles** and **thermophiles**. The optimum temperature for cellular metabolism is the result of the overall temperature optima of every enzyme contained within a bacterium. However, a different temperature optimum may exist for specific enzyme systems: for example in some bacteria the synthesis of a pigment may be favored at one temperature but not at another one.

Psychrophiles grow optimally below 20^{o}C and some grow well at subzero temperatures. Thermophiles grow optimally above 45^{o}C and some can grow at near boiling temperatures. Mesophiles grow best between 20^{o}C and 45^{o}. "Philism" is defined by where the optimal growth temperature occurs. Many bacteria can tolerate and even grow at temperatures much lower than their optimum temperature. For example some will optimally grow at 35^{o}C but can grow down to zero. This type of a bacterium would be described as a **psychroduric mesophile**.

Oxygen. **Obligate aerobes** are microbes which grow only in the presence of oxygen. **Obligate anaerobes** do not grow in the presence of oxygen, and **facultative anaerobes** are able to grow in the presence or absence of oxygen because they adjust their metabolism to oxygen: in oxygen they are respiratory while in anaerobic conditions they ferment. Another type of anaerobe with respect to oxygen is the **aerotolerant fermenter**: microbes that can tolerate oxygen but do not utilize it, instead they ferment both anaerobically and aerobically.

Obligate anaerobes usually require reducing conditions to grow and if the anaerobic condition has a positive redox potential they will fail to grow because their enzymes will not function unless reducing conditions are met. Anaerobic culture usually requires not only exclusion of oxygen but the inclusion of reducing agents such as thioglycollate which not only serves to remove oxygen but also reduces the redox potential of the culture medium. Redox indicators can be added to these media to detect the presence of oxygen. Methylene blue is commonly used: it is colorless when reduced and blue when oxidized. Another is resazurin which is colorless when reduced and red when oxidized.

Microaerophiles are oxygen requiring aerobes that can not tolerate high (air) concentrations of oxygen. They lack important enzymes that protect from the toxic effects of oxygen.

Two easily preformed tests, **Catalase test** and **Oxidase test**, are related to the use of oxygen. Cells growing in the presence of oxygen must protect themselves from the generation of hydrogen peroxide which occurs when two electrons (and two protons) reduce oxygen (refer to your text and see how super oxide dismutase is involved with life in air - the toxic aspects of oxygen!). **Catalase** is an iron containing protein that avidly binds first a molecule of hydrogen peroxide and then when it comes upon the next hydrogen peroxide it reacts the first with the other to form oxygen and water:

$$H_2O_2 + H_2O_2 \rightarrow 2\ H_2O + O_2 \uparrow$$

thereby eliminating hydrogen peroxide. In the catalase test a portion of the colony is taken and placed in a drop of 3% H_2O_2; the **generation of bubbles within a minute is a positive test** (Figure 1.).

Figure 1. Generation of bubbles is a positive catalase test

The **oxidase test** tests for the presence of cytochrome c. If a microbe has cytochrome c it will be able to oxidize the oxidase test reagent (tetramethyl-paraphenylenediamine) which is colorless to a blue color. The test reagent in the reduced state is colorless and in the oxidized state is deep blue. To do this test, a part of the colony is placed into a drop of the test reagent (on a slide), a **positive test is the change in the color of the bacteria to dark blue within a few seconds** (Figure 2.).

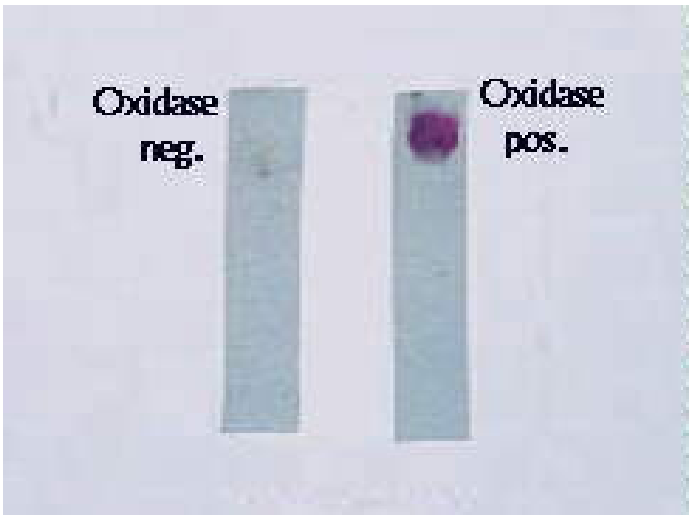

Figure 2. Change in the color of bacteria to dark blue is a positive oxidase test

Salinity. With respect to salinity (concentrations of NaCl) bacteria are classified as being either **extremely halophilic** (requires > 2M NaCl for growth), **moderately halophilic** (requires 0.5 to 3% NaCl for growth) and **nonhalophilic** (does not require NaCl for growth). **Halophilism** is both the requirement for a medium of high osmotic pressure and sodium ions. The sodium requirement can not be replaced by other cations although many halophiles also require magnesium. **Osmophilism** on the other hand is the requirement of a high osmotic environment for growth. This requirement can be fulfilled by many different osmotic agents (solutes) such as NaCl or sucrose. The halophilic and osmophilic physiologies reflect evolutionary adaption to naturally occurring environments that are highly saline or osmotic. Growth at high salt concentrations can be described as either halophilic or haloduric depending upon the ability of the microbe to grow in media lacking NaCl.

MATERIALS

1. 5 BHI Agar slants/group.
2. 1 Thioglycollate Agar Deep (16x150 mm tube, more than 3/4 filled), molten at 45^{o}C.
3. 1 Nutrient Agar Deep containing 0.002 gm Methylene Blue/liter, molten.
4. 1 BHI broth with 3% NaCl.
5. 1 BHI broth with 7% NaCl.
6. 1 BHI broth with 15% NaCl.
7. 1 BHI broth with 25% NaCl.
8. Catalase Reagent (3% H_2O_2).
9. Oxidase Reagent.
10. Cultures: BHI broth cultures of:

Serratia marcescens	*Vibrio harveyi*
Pseudomonas fluorescens	*Staphylococcus aureus*
Bacillus stearothermophilus	*Micrococcus roseus*
Streptococcus faecalis	

11. Fluid Thioglycollate culture of *Clostridium sporogenes*.

PROCEDURE

1. Each group (pair) will be given an organism and will inoculate it into a variety of media. Record the presence or absence of growth at the next lab period (2 days) and then one week later. Often growth at lower temperatures takes longer.

2. Inoculate 5 BHI slants. Incubate one each at 4^{o}C, 25^{o}C, 37^{o}C, 45^{o}C, and 55^{o}C.

3. Inoculate 5 BHI broth tubes containing media with 0%, 3%, 7%, 15% and 25% NaCl. Incubate at 37˚C.

4. The molten Thioglycollate and Nutrient Agars should be inoculated all the way to the bottom. Allow the agar deeps to solidify and incubate at the temperature given by the instructor (30˚C).

5. After optimum growth has been obtained on a BHI slant. Aseptically remove a bit of growth and place into a drop of the oxidase reagent on a slide. Observe for a few minutes or until the cells become blue, if they do not become blue within a few minutes the test is negative.

6. After performing the oxidase test, place a drop of the catalase reagent directly on the growth in the tube. Observe for the presence of gas bubbles, this is a positive test.

7. After recording your results, put your results with other groups of the class on the blackboard. How do your physiologically define each organism with respect to the environmental conditions required for growth?

QUESTIONS

1. Why is it that many anaerobes are catalase negative?

2. Is it possible to obeserve an aerobe showing a negative oxidase test?

3. How does thioglycollate work? What is the role of the dye in the thioglycollate and nutrient agar deeps?

RESULTS

Name of bacterium tested: _____

Growth (+ or -) at:

4°C ____	No NaCl ____
25°C____	3% NaCl ____
37°C____	7% NaCl ____
45°C____	15% NaCl____
55°C____	25% NaCl____

Growth in Thioglycollate and Nutrient Agar Deeps:

	Thioglycollate Agar	**Nutrient Agar Deep**
Color (Methylene Blue)		
Top	_____	_____
Middle	_____	_____
Bottom	_____	_____
Growth (Turbidity)		
Top	_____	_____
Middle	_____	_____
Bottom	_____	_____

Oxidase Test: Color:_____ Oxidase:_____ (positive or negative)

Catalse Test: Bubbles:_____ Catalase:_____ (positive or negative)