EFFECTS OF OSMOTIC PRESSURE ON MICROBIAL GROWTH

Principle and Purpose

Microorganisms are surrounded by a selectively-permeable, plasma membrane that helps sense environmental cues. The plasma membrane's function can be affected by osmotic pressure. This pressure is a result of different solute concentrations separated on opposing sides of the membrane. The membrane is only permeable to the solvent, i.e., the substance in which the solute is dissolved or suspended. In many biological systems, the solvent is usually water. The direction and rate of movement of water across the plasma membrane, in accord with the solute concentration, is the source of osmotic pressure.

Solute solutions can be designated as hypotonic, hypertonic, or isotonic (Fig. 1). Hypotonic solutions have low solute concentrations as compared to the cytoplasm of a cell. Thus, the natural consequence to have equal concentrations of solutes within and outside the cell causes water to cross the membrane into the cell. Hence, the cell would swell and burst unless there is a mechanim to prevent the membrane from expanding beyond its limits. In most microbes, the bursting of the cell is prevented by the presence of a cell wall. The rigidity of this structure maintains the shape of the cell and the integrity of the plasma membrane. Most common bacteria react in this manner to hypotonic conditions.

Under hypertonic conditions, the solute concentration is higher on the outside of the cell's plasma membrane compare to that in the cytoplasm. Therefore, water tends to flow out of the cell into the surounding environment causing the membrane to shrink away form the cell wall. This results in the dehydration of the cell, a condition known as plasmolysis. Hence, most typical bacteria would be affected in this manner by high solute (salt) concentrations in their environment. However, halophile ('salt loving') bacteria tolerate hypertonic environments. More so, some bacteria have adapted to live in extremely high salt environments and cannot remain viable in the absence of such conditions.

Finally, isotonic conditions occur when the solute concentrations are equivalent on both sides of the plasma membrane. Thus, the direction of water flow does not change drastically and the microbe is in osmotic equilibrium with its environment, i.e., the plasma membrane remains intact neither swelling or shrinking.

In the present exercise students will examine the effects of various salt concentrations on the growth of three bacteria - *Escherichia coli*, *Staphylococcus aureus*, and *Halobacterium salinarum*. The degree to which salt affects the growth of these microbes shall be observed subjectively (i.e., visually).

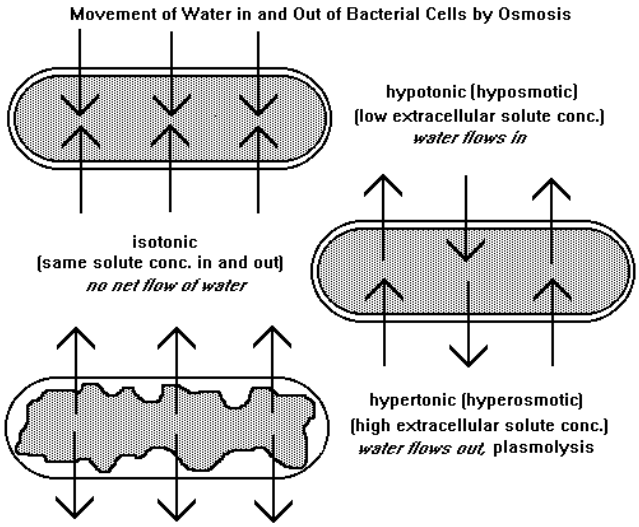

Figure 1. Solute concentrations and water movement (osmosis) on microbial cells. (http://microbiowiki.wikifoundry.com/page/Microbial+Nutrition+and+Growth)

Learning Objectives

Upon completion of this exercise, a student should be able to:

- Demonstrate the affects of osmotic pressure on bacterial growth; and
- Discern differences among bacteria based upon their tolerance of various salt concentrations, i.e., osmotic pressure.

Materials Required

The following materials are necessary to successfully conduct this exercise:

Organisms

- The following organisms should be provided as 24-48 hour-old TSB cultures:
  - *Escherichia coli* (ATCC 25922) [abbreviated as *E. coli*]
  - *Staphylococcus aureus* (ATCC 25923) [abbreviated as *S. aureus*]
- Salt broth culture of *Halobacterium salinarum* (NRC-1 [Carolina Biological]) [abbreviated as *H. salinarum*]

Materials

- Sterile cotton swabs
- Parafilm™
- Scissors
- TSA plate
- TSA plate with 0.5% NaCl
- TSA plate with 5% NaCl
- TSA plate with 10% NaCl
- TSA plate with 20% NaCl
- TSA plate with 25% NaCl

Procedures

Students shall review and use the BIOL 3702L Standard Practices regarding the labeling, incubation, and disposal of materials.

1) Obtain one of each of the TSA plates containing no (0%), 0.5%, 5%, 10%, 20% and 25% NaCl. Be sure to label each plate appropriately on the bottom (agar) side of the plate.
2) Using a marker on the bottom (NOT the lid) of dish, divide each plate into thirds. On each plate, label the one sector as "*E. coli*", another as "*S. aureus*", and the remaining section as "*H. salinarum*".
3) Aseptically dip a sterile cotton swab into the broth culture of *E. coli*, then gently press the swab to the interior portion of the tube to remove the excess fluid. Remove the swab, then wipe the sector of each plate labeled for *E. coli* in the following order: 0% NaCl, 0.5% NaCl, 5% NaCl, 10% NaCl, 20% NaCl, and 25% NaCl. Do not swab beyond the sector labeled for *E. coli*.
4) Repeat step 3 for the broth culture of *S. aureus* being sure to swab the respective sector of the various agar plates.

5) Repeat step 3 for the broth culture of *H. salinarum* being sure to swab the respective sector of the various agar plates.

6) Using scissors, cut a small strip of Parafilm™ and wrap/stretch it along the sides of each plate where the lid and the bottom parts join. Do not stretch the Parafilm™ across the face of the Petri dish lid.

Note: Due to the potential for prolonged incubation of the plates, the purpose of the Parafilm is to reduce evaporation.

7) Incubate all plates, inverted (portion of dish with the agar side up and lid on bottom), at 37°C for 48 hours.

8) Remove the labeled plates from the incubator. There should be no need to remove the Parafilm™ seal.

   Observe the amount of growth on each plate

Interpretation of Results: Use the following scale to record your observations: 0, no growth; +, little visible growth/turbidity; ++, some visible growth/turbidity; +++, moderate growth/turbidity; ++++, luxurious growth/turbidity. Use the degree of growth of *E. coli* on the agar plate without added NaCl as an example of '++++' growth.

   Record your observations on the report sheet attached to this exercise.

9) Growth of *H. salinarum* is often slow. Replace all plates in the 37°C (inverted) until the next laboratory period and make additional observations. Repeat making additional observations for any *H. salinarum* growth over the next week or two.

   Again, record your observations on the report sheet attached to this exercise.

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Student Name: ________________________________

COMPLETE THE FOLLOWING TABLE BASED UPON YOUR VISUAL OBSERVATIONS.

Use the following scale: 0, no growth; +, little visible growth; ++, some visible growth; +++, moderate growth; ++++, luxurious growth.

Organism	Degree of Growth:					
	‘control’ 0% NaCl	0.5% NaCl	5% NaCl	10% NaCl	20% NaCl	25% NaCl
Day 2						
Escherichia coli						
Staphylococcus aureus						
Halobacterium salinarum						
Day _____						
Escherichia coli						
Staphylococcus aureus						
Halobacterium salinarum						
Day _____						
Escherichia coli						
Staphylococcus aureus						
Halobacterium salinarum						
Day _____						
Escherichia coli						
Staphylococcus aureus						
Halobacterium salinarum						
Day _____						
Escherichia coli						
Staphylococcus aureus						
Halobacterium salinarum						

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Discussion Questions:

The following questions may require some background research. Be sure to cite your sources.

1. Which of the organisms used in this exercise is "osmotolerant"? What is a likely mechanism for this level of tolerance?

2. How role does osmotic pressure play in regard to the action of the drug penicillin against a penicillin-sensitive organism?

3. Explain why can a jar of grape jelly be left open on the kitchen counter for prolonged periods of time, yet no mold or bacteria will grow on it?