

TEMPERATURE AND SALT TOLERANCE

Principle and Purpose

Enterococci were once considered members of the genus *Streptococcus*. Both appear morphologically similar and share a few physiological characteristics. However, genome analysis has shown *Enterococcus* is a validly separate genus that exhibits some distinct differences in physiology from most species of *Streptococcus*. These include not only differences in salt tolerance (Fig. 1), but also distinct responses to growth temperatures. In the following exercise, students will use brain heart infusion (BHI) broth, with and without 6.5% NaCl, to distinguish between these two genera as well as compare it to the morphologically similar genera *Staphylococcus* and *Micrococcus*.



Figure 1. Salt Tolerance of *Enterococcus faecalis*. BHI broths with 6.5% NaCl were inoculated with *Streptococcus pyogenes* (left image) and *E. faecalis* (right image) and incubated for 24 hours at 35°C. Note the differences in turbidity. (https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/NaCl65Media.htm)

Learning Objectives

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

- Properly conduct the temperature/salt tolerance test;
- Accurately interpret the results of this test; and
- Discern between streptococci and enterococci.

Materials Required

The following materials are necessary to successfully conduct this exercise:

Organisms - The following organisms should be provided as 24-48 hour-old TSA slant or plate cultures:

- *Staphylococcus aureus* (ATCC 25923) [abbreviated as *S. aureus*]
- *Enterococcus faecalis* (ATCC 19433) [abbreviated as *E. faecalis*]
- *Streptococcus pyogenes* (ATCC 19615) [abbreviated as *S. pyogenes*]
- *Micrococcus luteus* (ATCC 4698) [abbreviated as *M. luteus*]

Media

- Brain Heart Infusion (BHI) broth
- Brain Heart Infusion + 6.5% NaCl (BHI/NaCl) broth
- Wickerham card

Equipment

- 45°C incubator

Procedure

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

- 1) Obtain ten (10) tubes of plain BHI broth and ten (10) tubes of BHI containing 6.5% NaCl (BHI/NaCl).
- 2) Label two tubes of each broth as follows: *S. pyogenes*, *E. faecalis*, *S. aureus*, *M. luteus*, and Control.
- 3) To one set of five (5) labeled plain BHI broth tubes, add the label '37°C'. Label the remaining set of five (5) plain BHI broth tubes as '45°C'. Be sure to include other information (e.g., date) as appropriate.
- 4) To one set of five (5) labeled plain BHI/NaCl broth tubes, add the label '37°C'. Label the remaining set of five (5) plain BHI/NaCl broth tubes as '45°C'. Be sure to include other information (e.g., date) as appropriate.
- 5) To the tubes of plain BHI and BHI/NaCl broths labeled for *S. pyogenes*, use a microbiological loop to *lightly* inoculate these tubes by aseptically transferring a small amount of growth from the respective culture provided.
- 6) To the tubes of plain BHI and BHI/NaCl broths labeled for *E. faecalis*, use a microbiological loop to *lightly* inoculate these tubes by aseptically transferring a small amount of growth from the respective culture provided.
- 7) To the tubes of plain BHI and BHI/NaCl broths labeled for *S. aureus*, use a microbiological loop to *lightly* inoculate these tubes by aseptically transferring a small amount of growth from the respective culture provided.
- 8) To the tubes of plain BHI and BHI/NaCl broths labeled for *M. luteus*, use a microbiological loop to *lightly* inoculate these tubes by aseptically transferring a small amount of growth from the respective culture provided.
- 9) The remaining tubes of BHI and BHI/NaCl broths labeled as 'Control' are NOT to be inoculated, but they are to serve as sterility controls.
- 10) Incubate all tubes labeled as '37°C' in the incubator set at 37°C for 36-48 hours. If screw-cap tubes are used, be sure that the lids are loose, but not so loose as to fall off.
- 11) Incubate all tubes labeled as '45°C' in the incubator set at 45°C for 36-48 hours. If screw-cap tubes are used, be sure that the lids are loose, but not so loose as to fall off.
- 12) Remove all the tubes from the incubators. Observe the degree of turbidity of each tube.

Note: To help discern the degree of turbidity of each tube, a Wickerham card (as shown in the background of Fig. 1) can be used to compare the degree of growth.

Score each using the following scale: 0, no growth; +, little visible growth/turbidity; ++, some visible growth/turbidity; +++, moderate growth/turbidity; +++++, luxurious growth/turbidity.

Note: Use the plain BHI broth inoculated for a given species and incubated at 37°C as an example of “+++” growth for all tubes incubated at either temperature. Use the uninoculated (hopefully still not turbid) plain BHI broth tube incubated at 37°C as an example of ‘0’ growth. Be sure to use the proper tube to score the appropriate species, i.e., use the plain BHI broth inoculated with *Staph. aureus* and incubated at 37°C as the “+++” culture to score all tubes, both plain BHI and BHI/NaCl, inoculated with *Staph. aureus* and incubated at 37°C or 45°C. For any given species, if growth is poor in the plain BHI broth tube incubated at 37°C for 48 hours, re-incubate all tubes for that species for an addition five (5) days (i.e., seven days total). Remove the tubes and again score the growth responses in terms of turbidity.

Record any observations on the data report sheet attached to this document.

Student Name: _____

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Record the degree of turbidity of each tube as follows: 0, no growth; +, little visible growth/turbidity; ++, some visible growth/turbidity; +++, moderate growth/turbidity; +++++, luxurious growth/turbidity.

Note: Use the plain BHI broth inoculated for a given species and incubated at 37°C as an example of “+++” growth for all tubes incubated at either temperature. Use the uninoculated plain BHI broth tube incubated at 37°C/45°C as an example of ‘0’ growth.

Growth Response of Selected Cocci to 6.5% NaCl at Different Temperatures

Incubation Temperature	<i>Streptococcus pyogenes</i>		<i>Enterococcus faecalis</i>		<i>Staphylococcus aureus</i>		<i>Micrococcus luteus</i>	
	BHI	BHI + NaCl	BHI	BHI + NaCl	BHI	BHI + NaCl	BHI	BHI + NaCl
37°C								
45°C								

Discussion Questions

- Based upon the results obtained in this exercise, speculate which species would grow on Mannitol Salt Agar.
- What is the mechanism by which enterococci are able to grow in high salt conditions? This question may require additional research. Be sure to cite your source(s).