Effect of Temperature on Gene Expression

Introduction

Explore the effect of temperature on the expression of specific genes in *Serratia marcescens* bacteria.

Biological Concepts

- Microbiology
- Gene expression
- Enzymes

Background

All living organisms contain many genes encoded in their DNA sequences. Each gene contains the information needed for the cell to produce a specific protein or enzyme required to carry out a particular biochemical function or to fulfill a particular cellular process. Factors such as pH, light, and temperature may cause certain genes to be "turned on" or to be "turned off" under certain conditions. In other words, although the gene is always present in the organisms DNA, a variety of stimulants or inhibitors may cause gene expression (the transcription of the gene DNA to produce mRNA) to start or stop, respectively. Ultimately, if gene expression is turned off, the appropriate mRNA is not produced and translation will not occur. Since the corresponding proteins are not produced, the cellular function controlled by that gene stops and the gene is no longer expressed.

In this activity, the study organism is *Serratia marcescens*, a Gram-negative common soil bacterium, which may be white, pink or red in color. The presence of specific enzymes within *S. marcescens* is required for the dark, red-colored pigment prodigiosin to be produced. Although the bacteria will grow and thrive over a fairly wide temperature range (17–43 °C), the gene for prodigiosin is only expressed in part of that temperature range. If the bacteria are grown at a temperature in which the enzymes responsible for the synthesis of prodigiosin are produced, the pigment will be present and the bacteria will appear red in color. Alternatively, when the bacteria are grown at a temperature in which the enzymes responsible for the synthesis of prodigiosin is not transcribed, no pigment is made and the bacterial colonies will appear white or colorless.

In this activity, *S. marcescens* will be grown on nutrient agar at room temperature (approx. 25 °C) and at human body temperature (37 °C). The effect of temperature on differences in gene expression will be defined based on the presence or absence of the prodigiosin pigment.

Materials

- *Serratia marcescens* culture
- Incubator
- Petri dishes containing nutrient agar, 2
- Sterile cotton swabs, 2
- Tape, masking
- Water, sterile
- Wax pencil

Safety Precautions

Although Serratia marcescens *is a non-pathogenic bacterium, there is always the potential for contamination by pathogens when dealing with microorganisms. Wear goggles and gloves when handling microbes, and always use sterile or aseptic techniques. Tape all Petri dishes shut and do not open the Petri dish lids after inoculation with bacteria. Sterilize all work areas and surfaces after handling the bacteria as instructed by your teacher and wash your hands with anti-bacterial soap before leaving the laboratory.*

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Procedure

1. Obtain two nutrient agar Petri dishes and a wax pencil. On the bottom of one of the dishes write 25 °C along with the names of the group members and the date. On the bottom of the second Petri dish write 37 °C again with the names of the group members and the date.
2. Carefully open the wrapper of a sterile cotton swab. Do not allow the cotton tip to contact any surface, including your fingers. Lightly touch the cotton tip to a container of sterile water so that it is moist but not saturated. Obtain a sample of *Serratia marcescens* by gently touching the wet cotton tip to the surface of the slant culture. Lift the lid of the Petri dish labeled 25 °C a few centimeters and gently streak the surface of the agar in a zigzag pattern using the tip of the cotton swab. See Figure 1. Immediately close the Perti dish lid.

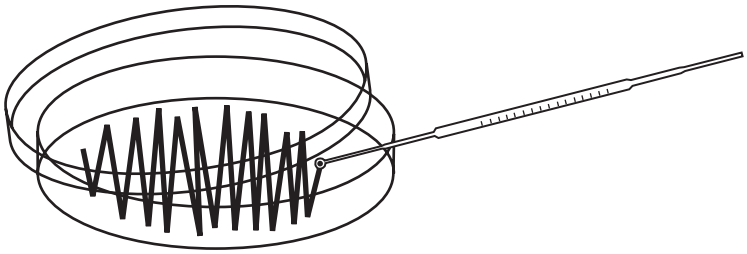

Figure 1.

3. Place the used cotton swab back into the original wrapper and throw it away in the trash.
4. Seal the Petri dish closed by wrapping masking tape around the sides.
5. Repeat steps 2 and 3 again using the 37 °C dish and a fresh cotton swab.
6. Store the 25 °C dish on a counter or table at room temperature and place the 37 °C plate in an incubator as directed by your teacher.
7. After 48 hours, check the Petri dishes and observe any differences in the appearance of the bacterial growth on the plates.
8. Disinfect the area per your teacher's instructions.

Teacher's Notes

Effect of Temperature on Gene Expression

Materials (for a class of 30 students working in pairs)

- Bleach solution, 10%, isopropyl alcohol, 70%, or Lysol solution
- *Serratia marcescens* culture, 1 tube
- Incubator
- Nutrient agar, 23 g
- Beaker or flask, 1-L
- Heat-resistant gloves
- Petri dishes, plastic, sterile, 30
- Sterile cotton swabs, 60
- Stirring rod or magnetic stirrer
- Tape, masking
- Water, sterile, 100 mL
- Wax pencils

Preparation

To prepare one liter of nutrient agar from powdered agar, heat one liter of distilled or deionized water close to a boil. While stirring, add 23 grams of nutrient agar powder to the hot water. Keep solution close to 100 °C but do not allow it to boil. When the solution turns clear, remove the beaker or flask from heat while wearing protective gloves. Allow the solution to cool for a few minutes before pouring it into the Petri dishes. Fill each sterile plastic Petri dish about one-half full and immediately replace the lid on the dish. Set the incubator to 37 °C a couple of hours before beginning the activity. Prepare either a 10% bleach, 70% isopropyl alcohol, or Lysol solution for disinfecting surfaces.

Safety Precautions

Although Serratia marcescens *is a non-pathogenic bacterium, there is always the potential for contamination by pathogens when dealing with microorganisms. Instruct students to wear goggles and gloves when handling microbes, and to follow aseptic techniques. Do not allow students to open the Petri dish lids after inoculation. Remind students to disinfect the work surfaces and to wash their hands thoroughly with soap and water after working with microorganisms.*

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures governing the disposal of biological waste. Upon finishing work with bacterial cultures, label the stock tubes, instruct students to disinfect their work areas (including the incubator handle). Use a spray bottle containing a 10% bleach solution (if the surfaces are compatible with bleach), a 70% isopropyl alcohol solution or Lysol solution to sterilize all work surfaces. Microbiological cultures may be disposed of according to Flinn Suggested Biological Waste Disposal Method Type I.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12
Systems, order, and organization
Evidence, models, and explanation

Content Standards: Grades 5–8
Content Standard C: Life Science, structure and function in living systems, reproduction and heredity, regulation and behavior, diversity and adaptations of organisms

Content Standards: Grades 9–12
Content Standard C: Life Science, molecular basis of heredity, organization in living systems

Tips

- The "25 °C" dishes may be stored at room temperature, which is generally in the range of 20–25 °C. If preferred, the exact temperature of the room may be measured and written on the dish rather than 25 °C.
- Cultures grown at room temperature should have smooth, dark red bacterial colonies present. Cultures grown at 37 °C should display white or pale pink colonies. To achieve all white colonies, subculture the 37 °C Petri dish onto a fresh Petri dish and incubate at 37 °C for 48 hours.
- Stock cultures of *S. marcescens* contain red-colored colonies. However, if the bacteria are shipped in hot weather they may be pink or white upon arrival. If you do not want students to see the original color of the bacteria, place black electrical tape around the tube and dip the sampling swabs into the tube for the students.
- As an alternative or follow-up procedure, students may experiment with growing *S. marcescens* cultures at different temperatures between room temperature and body temperature to determine the temperature cut-off for the expression of the red pigment. Cultures grown at temperatures in between those tested in this activity are often pink in color.

Materials for *Effect of Temperature on Gene Expression* are available from Flinn Scientific, Inc.

Catalog No.	Description
LM1013	Serratia marcescens
AB1227	Swab Applicator, Sterile, pkg. 200
FB0526	Nutrient Agar Prepared Plates, 10
AP8170	Petri Dishes, Plastic, Sterile, pkg. 20
AP1565	Incubator

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.