Does Sunscreen Protect my DNA?

Abstract

In this laboratory experiment students explore how effectively different sunscreens protect yeast cells from damage caused by exposure to ultraviolet (UV) radiation.

Learning Objectives

- In this laboratory experiment students explore how effectively different sunscreens protect yeast cells from damage caused by exposure to ultraviolet (UV) radiation. Students may compare differing SPFs of the same brand of sunscreen or different brands with the same SPF. The experiment utilizes a strain of yeast that lacks several DNA repair mechanisms; UV-induced mutations lead to cell death. The experiment provides data for students to make relative comparisons between sunscreens.

Estimated time

- Class time 90 minutes
- Prep time 60-120 minutes

Materials

- UV-sensitive yeast strain*
- YED (Yeast-Extract Dextrose) media plates - 1 to grow yeast, and 1/student or student pair [NOTE: You can find instructions for preparing your own agar plates at https://teach.genetics.utah.edu/content/microbiology/plates/.]
- Sterile container with lid (one for each class)
- Sterile pipet to measure 1 ml
- Pipet bulb or pipet pump to use with the pipet, if needed
- Sterile test tubes with caps (optional)
- Sterile distilled water
- 70% or 95% ethyl or isopropyl ("rubbing") alcohol
- Waterproof marking pens with fine tips - 1/student pair
- Sterile, flat toothpicks
- Aluminum foil
- Paper towels
- Self-sealing ("Zip-lock") plastic bag
- Transparent ("Scotch") tape
- Small brown paper bags (optional)

* This strain of yeast has mutations in an excision repair gene (rad1), an error prone repair gene (rad18) and a photoreactivation repair gene (phr1).

- Sunscreens of different SPFs and/or different brands
- Other sun protection items such as lip gloss, lenses from sun glasses and fabric
- 2-inch squares of dark paper such as construction paper
- Source of UV radiation – sun, quartz halogen or fluorescent light (details in a section following)
- Sun protection - UV-protective glasses, hats, sunscreen, etc. (needed if using the sun as your UV radiation source)
- Place to incubate the yeast (30 degrees C incubator or room temperature)

Background Information

Before carrying out this experiment, students minimally need to understand that DNA contains the instructions for proper cell function; DNA can be changed so that cells no longer function normally, such as in cancer, which is uncontrolled cell growth; skin cancer and other adverse health effects can be caused by exposure to ultraviolet (UV) radiation, which comes from the sun; and the meaning of sun protection factor (SPF) ratings on sunscreens.

Instructions

1. Immediately before the activity, prepare a visibly turbid suspension of UV-sensitive yeast cells in sterile water.

- Use the rounded end of a sterile toothpick to collect a small amount of yeast from the YED plate.
- Place the yeast on the side of a sterile container and add sterile water; you will need 1 ml of water for each student or pair.
- Replace the lid on the container and swirl it to mix the yeast and water. Add more yeast cells if necessary to make a visibly cloudy suspension of cells.
- You may choose to aliquot 1 ml of yeast solution for each student or pair into sterile, capped test tubes or dispense the yeast yourself.

NOTE: Yeast cells do not survive long in water; it is best to prepare a fresh yeast suspension immediately before each class.

2. Have students set up their experiments and expose the plates to UV radiation.

3. Incubate the yeast plates at 30 degrees C for 1-2 days or at room temperature for several days until a lawn of white yeast has grown on the plates.

- The plates should be incubated upside down and kept in the dark (incubator, in a drawer or closet, or covered with aluminum foil).
- If students are unable to look at the plates after the lawn of yeast is visible, the plates can be stored upside down in sealed plastic bags in a refrigerator.

4. After a lawn of yeast has formed on the plates, students collect and analyze their data, dracon-clusions and report their results. Photographs of sample results are available at http://www.phys.ksu.edu/gene/photos/solaruv.html

Tips and Tricks

- Cleaning with alcohol provides some sterilization and removes possible sources of contamination from dust, molds, etc.
- Remind students to write in small letters around the bottom edge of the Petri dish so that they can later see the results of their experiments. Depending on the products available, students may choose to compare different SPFs of the same sunscreen, different sunscreens of the same SPF, or other differences among products. They may also use other sun protection items such as lip gloss, fabric, or sunglass lenses (an optician may be willing to provide you with "blanks", which are lenses that have not been shaped to fit particular frames, or lenses from old glasses).
- Swirl the container of UV-sensitive yeast to re-suspend the cells before removing each sample. Lift the lid of the Petri dish only enough to add the yeast suspension near the center of the agar. Keep the dishes closed as much as possible to reduce contamination.
- Place the media plates in a dark location such as a cupboard, drawer or brown paper bag. If you plan to expose the plates to UV radiation by holding them perpendicular to the sun, it is particularly important that all of the water from the yeast suspension be absorbed by the media before continuing with the next step of the experiment. If the water is not absorbed, the yeast cells will migrate across the surface of the plate when it is tilted, obscuring differences between treatments. If the plates will remain horizontal while being exposed to lights, it is not as crucial that all of the water be absorbed, although it is preferable.
- Transparent tape absorbs UV radiation so it should not extend onto the top of the Petri dish. Taping the two halves of the dish together keeps them from accidentally coming apart and allowing contamination to enter, and preserves the labeling for each treatment.
- Try to use equal amounts of sunscreen for each treatment; for more precise comparisons, use a balance to weigh each sunscreen amount. SPF testing is conducted using 2 mg per square centimeter of skin.
- Hold the dishes so the surface of the agar is perpendicular to the sun's rays. Placing a paper or card under the dish to show a shadow may be helpful; when the dish is perpendicular to the sun, the shadow will be smallest. You could also use a quartz halogen light, or a germicidal lamp as a UV source. (See Sources of Ultraviolet Radiation for exposure times.)
- If you place the dishes in a dark location, such as a cupboard or drawer, you will not need to cover them with aluminum foil.
- Placing the dishes upside down prevents moisture from collecting on the yeast.
- For optimal growth, the plates must be aerobic. Do not tightly seal them in plastic bags while they are incubating. You may find it helpful to incubate them in open food-storage bags, which keep them from drying out too quickly and protects them from contamination.
- Yeast strains can be stored on media plates in the refrigerator for up to six months or a year.
- Remove water condensation from the lids of media plates BEFORE turning them over by removing the lid, turning it over, and briskly flicking it. Or wipe with a sterile tissue. Then replace lid and turn over.

- To sterilize a container, place the cover or aluminum foil loosely over the mouth and use one of the following sterilization methods: autoclave or pressure cook for 15 minutes at 15 pounds of pressure or bake at 320oF for 2 hours.
- Toothpicks in unopened boxes are sterile and can be used directly from the box. Use scissors to cut a small hole (about 1/4 inch across) in one corner of the box to serve as a dispenser. The toothpicks in the box are pointing in both directions; use only the ones you grasp by the pointed end (leaving the rounded end sterile, for use).

Sources of UV radiation:

- Sunlight – For maximun exposure and effectiveness, hold plates of yeast so that the surface of the agar is perpendicular to the sun's rays. Placing a piece of paper or 3x5" card behind the Petri dish to show a shadow may be helpful; when the dish is perpendicular to the sun, the shadow will be smallest. To determine the number of minutes to expose the plates, consult "A Classroom Guide to Yeast Experiments" at https://www.phys.ksu.edu/gene/chapters.html.
- Quartz-halogen bulbs – Quartz-halogen bulbs emit UV radiation which is very similar to that reaching the earth's surface from the sun. These bulbs are used in some decorative lamps and work lights. Lights with these bulbs have a piece of glass over the bulb to absorb the UV radiation. If you remove the glass, these bulbs can be used as a source of artificial sunlight. Set the light up in a way that minimizes UV exposure. This includes placing the light below students' eye level and directing the light downward onto the surface where you place the yeast plates. Because quartz-halogen bulbs put out considerable heat, place the plates of yeast at least 20 cm (8 inches) from the bulb so as not to kill the yeast, warp the plastic Petri dishes, or melt the agar.

  There is considerable variation among bulbs in the amount of UV radiation and heat they produce. Before using this light source with students, test your bulb to determine the length of exposure time needed to kill most of the yeast. In our tests, exposure times have ranged from three to six minutes with 300W and 500W bulbs.

- Fluorescent lights – Fluorescent light tubes are filled with a mercury through it. Most fluorescent tubes are coated on the inside with a fluorescent material that absorbs this UV radiation. However, other types of fluorescent coatings can cause these tubes to emit each of the three types of UV radiation. UVA tubes are used as "black lights". UVB tubes are used to fluoresce the ethidium bromide dye used to stain DNA fragments in DNA electrophoresis. UVC tubes have no fluorescent coating and are often called germicidal lamps.

  Instructions for constructing a UV radiation chamber that utilizes fluorescent lights are available at http://www.phys.ksu.edu/gene/RAD.html.

  The wavelengths of light emitted by the fluorescent tubes is strongly temperature dependent; allow the lights to warm up for 30 minutes before use. Before using each type of bulb with students, test it to determine the length of exposure time needed to kill most of the yeast; 15-20 seconds is usually adequate for UVC.