# How much is too much sun?

## Investigations using UV-sensitive yeasts

Over the last decade the incidence of skin cancer in Scotland (specifically malignant melanoma) has increased by a staggering 30% [1]. The increased occurrence of this type of cancer can be attributed mainly to the rise in the number of Scots taking holidays abroad each year. A significant factor, particularly amongst younger people, is the use of sun-beds to achieve that healthy glow [2]. It is therefore important to ensure that our young people are informed of the dangers of too much sun or the over-use of sun-beds. We describe a practical activity which can be used to underpin this message.

## Background

When UV radiation hits living cells it may damage the DNA of the cells causing mutations. However, most cells can switch on repair mechanisms to deal with the mutations induced by UV radiation. Repeated damage to the DNA increases the chance of mutations being missed by these cellular repair systems. In humans this failure to repair may result in wrinkles, damage to the immune system and skin cancer.

DNA repair genes are found in normal baker's yeast (*Saccharomyces cerevisiae)* and have a similar function to those found in human cells. Some strains of yeast have mutations which prevent them from making certain repairs such that they then die when exposed to UV radiation. The commercial availability of UV-sensitive yeasts thus provides us with a reliable tool for use in the classroom to study the effects of this type of radiation on cells.

## Preparing for the Activity

Prior to this investigation, Yeast Glucose Agar (YGA) plates need to be prepared. YGA is a nutrient-rich medium that provides everything yeast needs to grow. The number of plates required will depend upon how the experimental protocol is adopted.

A UV-sensitive strain of yeast is available from Blades Biological (www.blades-bio.co.uk/); tel: 01342 850242). Stock plates of the yeast should be prepared from the initial culture by streaking and incubating at room temperature for 4-5 days or in an incubator at 30°C for 2-3 days. Plates should be wrapped in aluminium foil to reduce exposure to light during this phase. Using aseptic technique, a single colony from a stock plate should be suspended in 10 $cm^3$ sterile water and this suspension used as the starting point for a serial dilution of the organisms [3]. It is important to ensure that the bottles are covered in aluminium foil to protect the yeast cells from light. Serial dilution is necessary to ensure an appropriate number of colonies for counting will be obtained on the final plates (Figure 1).

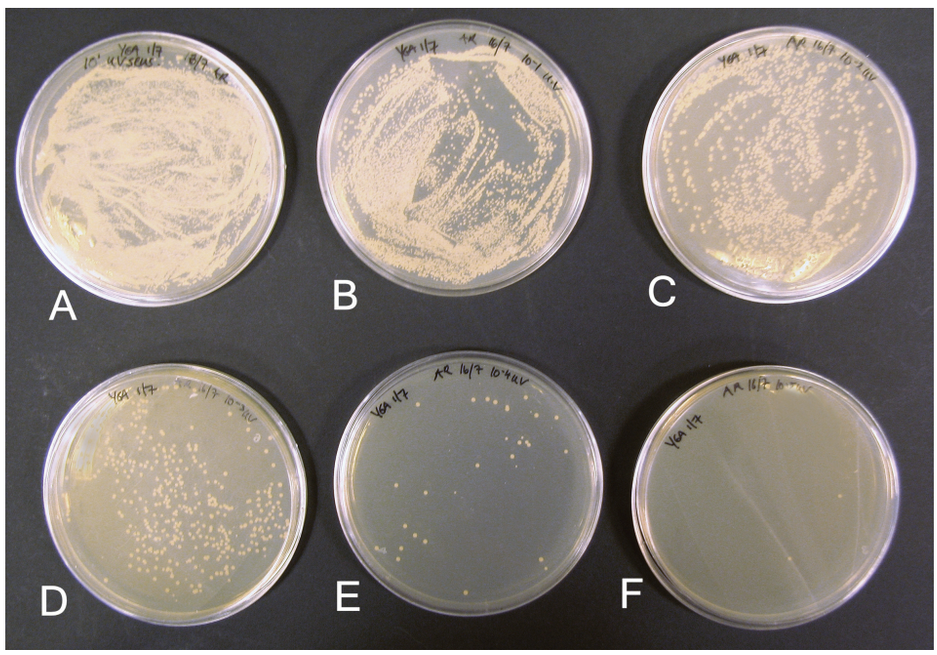


***Figure 1*** *- Serial dilutions of UV-sensitive yeasts showing the reduction in colony numbers. A = stock solution; B = $10^{-1}$ dilution; C = $10^{-2}$ dilution; D = $10^{-3}$ dilution; E = $10^{-4}$ dilution; F = $10^{-5}$ dilution.*

We found that a dilution of $10^{-4}$ provided a countable number of colonies. Pupils could carry out their own serial dilutions, particularly if they are using this technique for an AH Biology investigation.

The light source that we used initially was the sun. We found that on a bright, sunny day in the middle of summer, 15 minutes exposure time was enough to kill off most, if not all, of the mutant yeast cells. However, on cloudy or autumnal days the plates required 40-45 minutes exposure for cells to be substantially affected by UV. If the experiment is carried out in winter months it may be necessary to expose the yeast cells to a UV light source (we used a BDH lamp Ref. no. VL-315.BL, wavelength 365 nm). During all experiments, the lids were left on the Petri dishes since UV light (365 nm) is transmitted through plastic. Taking the lids off the dishes to expose the cells to UV is not recommended since we found that contamination of plates occurred when exposure times had to be longer.

## Carrying out the Activity

Prior to, and after carrying out the activity students should wash their hands well with soap and water and clean their work areas thoroughly using 1% bleach or Virkon™ and should use aseptic technique throughout [4]. Groups of pupils could be provided with a bottle of a $10^{-4}$ dilution of yeast cells. Using a sterile disposable pipette, 0.25 $cm^3$ of the suspension is removed from the bottle, ensuring that the neck of the bottle is flamed before and after removal. The suspension is transferred to the YGA plate and spread across the agar using a disposable spreader (reusable glass spreaders must be dipped in alcohol and flamed before and after).

We would advise that each group spreads a minimum of four plates. Plate 1 is not exposed to light, remaining covered in foil, and acts as a control. Plates 2, 3 and 4 are replicates for a given exposure time[1].

The foil is removed and the plates are exposed to light for a given length of time, depending upon the lighting conditions. Different groups would expose their plates for different lengths of time. The plates should then be re-covered with foil and incubated at 30°C for 2-3 days, or at room temperature for 4-5 days, after which time the numbers of colonies in each plate can be counted and compared (Fig 2a and 2b).

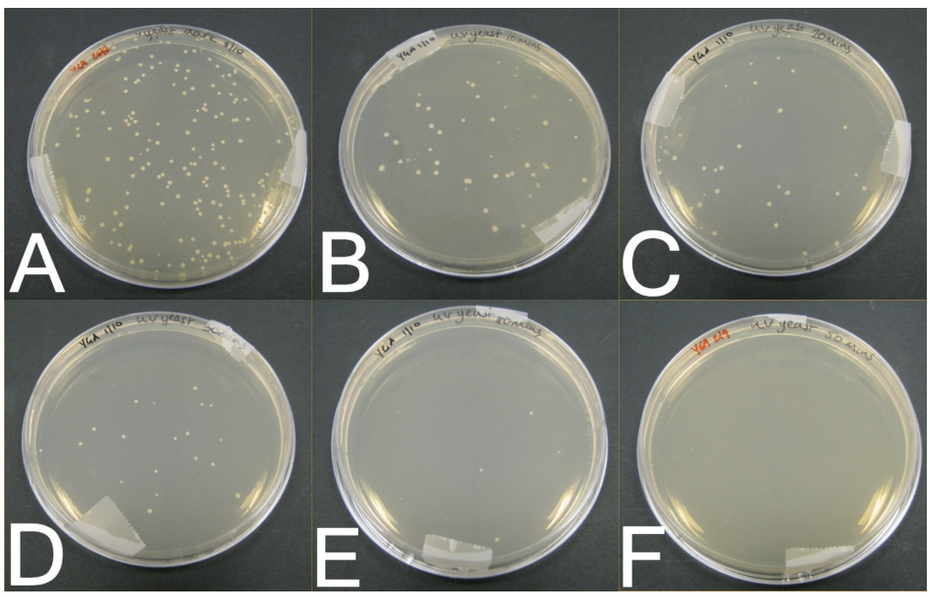


***Figure 2a*** *– UV-sensitive yeast exposed to sunlight for different lengths of time (colony counts were made after 2 days incubation at 30°C). A decline in numbers is observed as exposure time increases. A = time zero; B = 10 min exposure; C = 20 min exposure; D = 30 min exposure; E = 40 min exposure; F = 50 min exposure.*

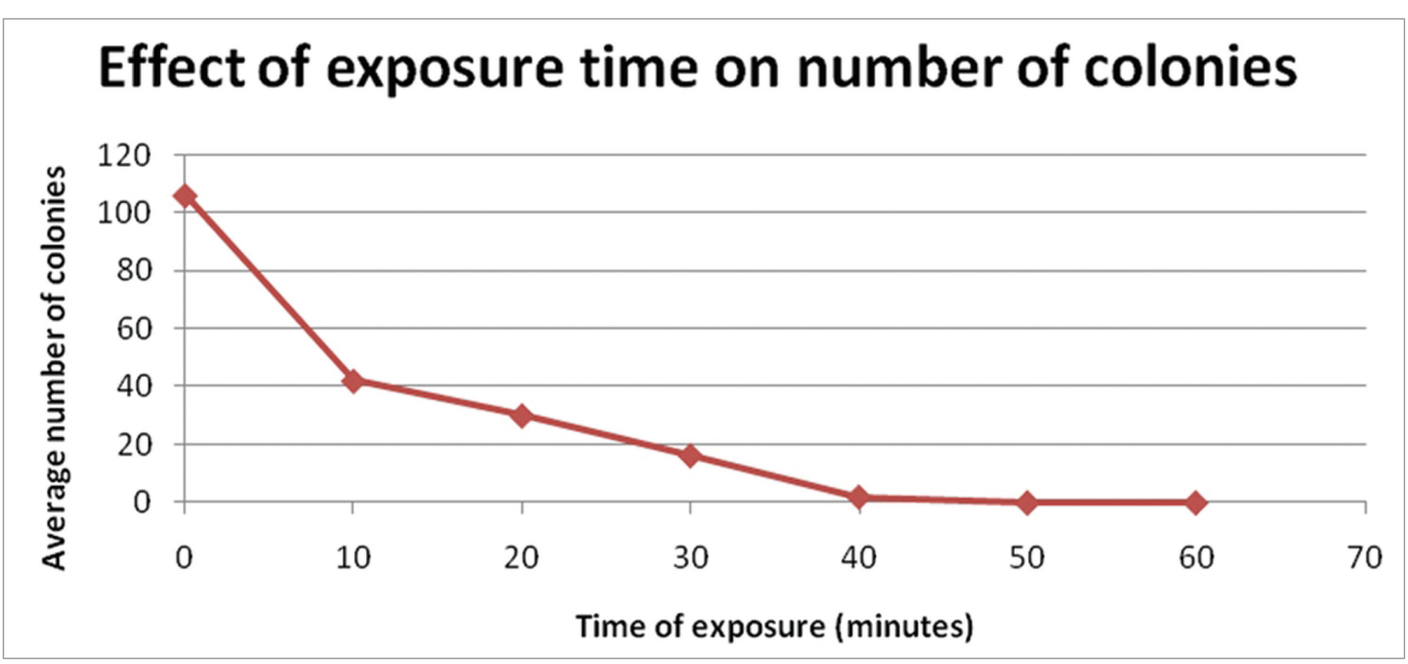


***Figure 2b*** *– Graphical representation of the data in Figure 2a. Sunlight was used as the source of light. Qualitatively similar results were obtained using a UV lamp emitting at 365 nm.*

[1] An attempt was made to divide plates into four sections, using one as a control and the others as experimental sections. However, it was felt that this did not allow a fair comparison between sections as the yeast suspension did not always appear to be evenly spread.

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## Applications

This technique can be used to investigate the effects of the following variables on the number of yeast colonies:

- Length of exposure to UV source
- Normal yeasts (e.g. baker's yeast) versus mutant strain
- Different Sun Protection Factors (SPF) of sunscreen (Figure 3), including lotions and oils with no SPF
- Different brands of sunscreen
- Do old bottles of sunscreen work as well as new ones?
- Different types and colours of fabrics
- Different sunglasses

It can be clearly seen from the results that as exposure time increases, the number of colonies growing after 2 days decreases substantially.

If sunscreens are to be applied, 0.2 g of sunscreen is sufficient to coat the surface of the lid. This can be spread out using a paper towel and removed once the plate has been exposed to the requisite amount of sunlight. It was found that, if left on for several days, the sunscreens clouded the lid and made observation of the colonies more difficult.

Preliminary studies looking at the effects of sunscreens containing different SPF indicate that the use of sunscreens increase the number of colonies growing after one hour exposure to UV light, but this effect depends on the SPF factor of the cream (Figure 3). Several trials have indicated that Factor 25 makes very little difference compared to the use of Factor 15. However, comparison with higher factors e.g. SPF 50 would be useful to investigate if this level of protection produces a substantial difference.

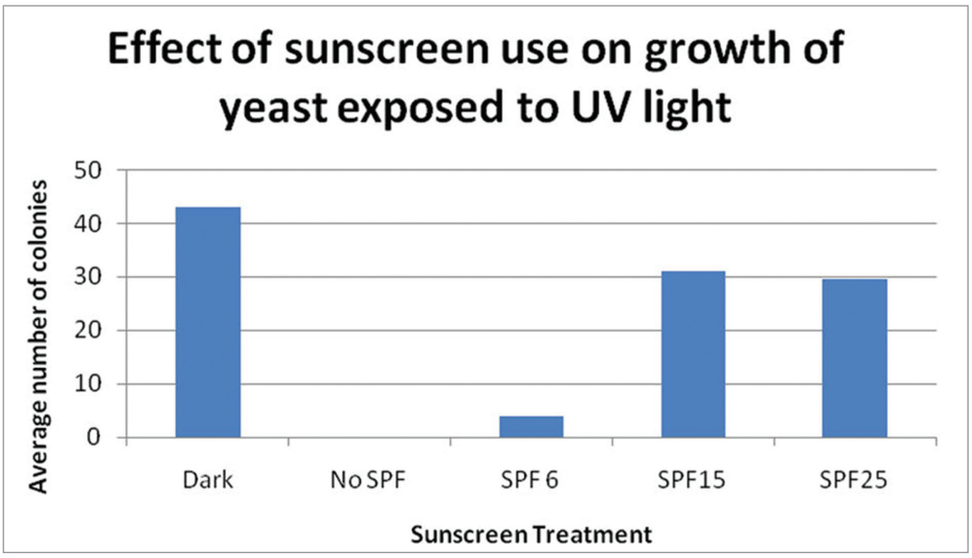


***Figure 3*** *– Plot showing the effect of sunscreens with different SPF values on the number of colonies (after 2 days incubation at 30°C) after exposure to UV light. Sunscreens were applied to the lids of the Petri dishes before irradiation. The control plate was not exposed to UV light.*

This technique lends itself extremely well to an AH Biology investigation or as part of a wider piece of work looking at the harmful effects of the sun and sun-beds. In terms of Curriculum for Excellence the activity might be used to support outcomes in Topical Science, encourage manipulative and investigative skills, and raise awareness about an important health issue.

## References


1. http://isd.scot.nhs.uk/isd/1048.html (accessed 15th April 2009)
2. http://info.cancerresearchuk.org/news/archive/pressreleases/2006/may/151734 (accessed 15th April 2009)
3. http://www.sserc.org.uk/members/SafetyNet/Microbio2/Documents/Techniques%20Cards.pdf (accessed 15th April 2009)
4. http://www.sserc.org.uk/members/SafetyNet/Microbio2/AT/PrepSW.htm (accessed 15th April 2009)