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Abstract

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Ultraviolet light is one of the most widely studied mutagenic agents. Its mechanism of action has been demonstrated to be a multiple one, consisting of both the formation of photoreversible thymine dimers within the DNA molecule and other, non-reversible alterations of the molecule. This experiment will be carried out both in the dark, thus avoiding photoreactivation, and in the light. When ultraviolet irradiation is carried out in the presence of white light, a considerable portion of the genetic damage induced is repaired through the photoreactivation process.

The second half of the experiment is carried out in almost complete darkness, so it is essential that the students plan and organize the work in advance. Equipment which will be needed during the blocked-out phase should be laid out on the work table in a logical order so that it will not be misplaced.

The student will be furnished a Neurospora culture which has a heavy conidial growth. Suspend the conidia in sterile water by tapping the side of the tube with the index finger. Filter the suspension through a very thin sterile cotton pad into a centrifuge tube to remove the mycelium. Centrifuge the filtered conidial suspension for 10 minutes. Pour off the supernatant and wash the conidia with sterile water, centrifuge and resuspend in sterile water. Determine the concentration of the conidia with a haemocytometer and adjust with sterile water to 5×10^6cells/ml. Irradiate the cells in a petri dish at 50 cm from a 15-watt germicidal lamp for 5 minutes, agitating the suspension constantly while irradiating. Withdraw aliquots at 0, 30, 60, 90, 120 and 300 seconds, diluting each aliquot l0x with Neurospora minimal broth (Difco). Thus, each sample will have been diluted 10 times upon withdrawal from the dish. The platings of each sample, for scoring of survivors, should be as follows: plate 1 ml of the following dilutions, $1 \times l0^{-4}$, $5 \times l0^{-3}$, 1×10^{-3}, 5×10^{-2}, 1×10^{-2}, 1×10^{-2} and 1×10^{-1}, respectively for the exposure times given above. Pipette the sample into a sterile petri dish and add 10 ml of Neurospora agar (Difco) which has been supplemented with 8 g of sorbose to induce colony formation. The agar should be liquid but not painfully hot to the touch and should be adequately swirled. Incubate the plates right side up at room temperature.

The procedure above should be followed during the second phase of the experiment, except that the lab should be made completely dark to guard against photoreactivation. A yellow safelight bulb may be used, if necessary and if kept at least five feet from the work area.

Construct a graph of survival rate against length of irradiation for Neurospora in both light and dark. Discuss the photoreactivation effect and the effects of ultraviolet light as a mutagenic agent.

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