ULTRAVIOLET MUTAGENESIS OF MOSS CELLS IN VITRO

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SUMMARY: A method using ultraviolet light as a mutagen for moss cells in tissue culture is presented. This method allows for the selection of mutants in the green plant, *Physcomitrella patens* (Hedw.) BSG, by varying selection techniques to fit the mutants desired and for studying the effect of ultraviolet on a green plant system. The ease by which the system can be manipulated makes it attractive for research, classroom, or demonstration needs.

Key words: tissue culture; moss; ultraviolet light; mutagenesis; *Physcomitrella patens.*

I. INTRODUCTION

The bryophytes as a group represent a useful tool for studying genetics of higher plants. The bryophytes are multicellular, physiologically and developmentally complex organisms with a wide range of morphological and physiological traits that are characteristic of green plants. A number of laboratories have isolated mutants of bryophytes (1-4).

The isolation of mutants may be enhanced by using some form of mutagenesis prior to selection for the desired class of mutants. The following procedure outlines the guidelines for treatment of moss vegetative cells with ultraviolet light. The experimental organism is *Physcomitrella patens* (Hedw.) BSG, a moss of the Funariaceae. It was originally isolated from material collected in Gransden Wood, Huntingdonshire, England by H. L. K. Whitehouse. The culture is clonal in that it was originally isolated from a single spore.

II. MATERIALS

A. Equipment

Ultraviolet light source, Ultraviolet Products Inc. PC0X1 from Fisher Scientific[1]
Constant temperature environmental chamber with fluorescent lighting, Percival No. E-54U[2]
Laminar flow hood, Baker Edge Gard, Baker Co.[3]
Plastic shield, 14-293-40[1]
Magnetic stirrer, 14-493-120MR[1]
Spectral radiometer, Ultra-violet Products J-2601[1]

B. Medium

Modified Knops medium (5)
50 ml liquid medium in 125 ml flasks
Solid medium, 20 to 25 ml, in petri dishes (medium for controls will contain no additives; but for purposes of selection for a specific class of mutants, selective agents such as antibiotics, toxins, and analogs may be added to the medium.)

C. Glassware and other

Blender, 14-509-17[1]
Blender container, 14-509-18A[1]
Erlenmeyer flasks, 125 ml, 10-040D[1]
Coil cotton, 25 mm, 07-885D[1]
Aluminum foil, grocery store
Petri dishes, 15 × 150 mm, plastic, 08-757-14[1]
Petri dishes, 20 × 150 mm, glass, 08-747F[1]
Magnetic stir bars, 14-511-66[1], or paper clips
Nitex cloth, 64 μm pore size, Tetko Inc.[4]
Glass funnels, 10-346-5A[1]
Long forceps, 10-316A[1]
U.V. safety glasses, 11-403[1]
Clock or watch with second hand
Parafilm, 13-374-5[1]
Sterile wide mouth pipettes plugged with cotton, 13-671-108A[1]
Pipetting device, 13-683B[1]

D. Moss cultures

Cultures may be obtained from Dr. Karen Hughes, Department of Botany, University of Tennessee, Knoxville, TN 37996-1100.

III. PROCEDURE

A. All procedures should be performed under sterile conditions in a laminar flow hood. Use

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standard sterilization techniques for all glassware, media, and other materials.

B. Preparation of cells for mutagenesis from blended vegetative cell colonies

1. Use established cultures of moss. Cultures growing in liquid medium or on agarized medium may be used (5).
2. Place 50 ml of sterile medium into the small blender container (sterile). If cultures from solid medium are used, remove the leafy gametophytes from the medium with forceps and place in the blender container. If liquid cultures are used, filter the plant mass from the liquid, collecting the material on sterile nitex cloth supported by a funnel. Place the material in the blender. Cap the blender container.
3. Blend the moss and medium in the blender for approximately 40 s, varying the speed from slow to fast and back. The resulting slurry will contain cells of various sizes, usually 4 to 5 cells, and cell debris.
4. Filter the slurry by pouring it into the nitex cloth supported by a funnel. Wash the material with 50 to 100 ml of sterile medium to remove the cellular debris.
5. Resuspend the material collected in 50 ml of sterile medium. If fragments of equal size are desired, the mixture may be filtered through nitex cloth of larger pore size. The number of cells may be determined by use of a hemocytometer. Alternately, an aliquot may be plated onto a petri dish and counted under a dissecting scope.
6. Pipette a known aliquot of the cell suspension (usually 25 ml) into a petri dish that contains a stir bar. Replace the cover on the dish.

C. Ultraviolet treatment

1. Arrange the ultraviolet light source above the magnetic stirrer in a sterile hood. We arrange the light source so that it is 6 in. above the surface to be irradiated. Shield the operator from UV light with heavy plastic or glass. The operator should wear UV opaque safety glasses and gloves to prevent UV exposure to eyes and skin. Adjust the radiation dose at the surface of the stirrer to an approximate fluence rate of 20×10^{-6} W/cm^2 at 254 nm. The fluence rate may be determined by a spectral radiometer sensitive to UV wavelengths. The fluence rate may be adjusted by altering the distance between the light source and the magnetic stirrer. If a radiometer is not available, an initial experiment may be carried out to determine a dose versus survival curve using procedures outlined below. A dose giving survival of approximately 35% may be used for further studies.
2. Place the petri dish on the magnetic stirrer and turn the stirrer to low speed.
3. Turn off all hood and room lights. UV mutagenesis must be done in the dark.
4. Turn on the UV light source. Remove the glass cover of the petri dish and begin timing the irradiation. Stop timing when the glass cover is replaced. Mutagenesis is varied by change of exposure to UV. Since glass is largely opaque to UV irradiation, removal and replacement of the glass cover is a convenient way of controlling the dose. At this fluence, we recommend an exposure time of 60 s or less. (A 60 s exposure gives an approximate survival of 35%. Survival at 2 min is 11%; at 5 min, 9%; and at 8 min, 1%.
5. Pour a thin layer of medium and cells from each of the irradiated cultures onto petri dishes containing appropriate medium for controls and the selection of mutants. Make sure cells are in suspension while pouring. Each 25 ml of mutagenized cells will cover approximately 5 to 10 plates of agarized medium.
6. Seal the plates with parafilm and wrap the plates tightly in aluminum foil to prevent light exposure, which will induce mutation repair in mosses (6).
7. Store the foil-wrapped plates in a growth chamber for 48 h. Our studies indicate that this period of time is sufficient to prevent most light activated DNA repair (6). After 24 h, remove the foil and place the plates back in a lighted growth chamber. At this point, the number of cells per plate may be counted with the aid of a dissecting scope.
8. Cells may be scored for survival (as assayed by induction of protonemal filaments from cells) at 1 or 2 wk. Mutant colonies may be isolated after 3 to 4 wk of growth; however, many mutants exhibit very slow growth and colonies may appear on selective medium even later.

IV. DISCUSSION

Ultraviolet light has been demonstrated to be a useful mutagen when error-prone postreplication repair is allowed to take place; thus every effort should be made to prevent other forms of

repair from occurring. Photoreactivation repair (light induced enzymatic repair) has been demonstrated in *Physcomitrella patens* (6); however, by preventing exposure to white light for 48 h, the time necessary for one round of cell division to take place, photoreactivation can be prevented. Even though excision repair still occurs, the reduced level of repair allows the error-prone repair processes to occur as well as the establishment of mutants. Mutants have been isolated after treatment with UV light from cells of *P. patens* (3).

The simplicity of UV mutagenesis with moss cells makes it an attractive tool for student or research needs. In addition, the moss system has many advantages as an organism for genetic studies in that it is haploid, has a short generation time, and can be grown under controlled conditions in culture. Care should be taken to minimize UV exposure to eyes and skin. We routinely shield the UV source from the operator with a sheet of heavy plastic or glass, wear gloves for manipulations, and wear glass safety glasses.

V. REFERENCES

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6. Nakosteen, P. C.; Dodson, D.; Hughes, K. Regeneration and photoreactivation after ultraviolet irradiation of moss cells. Environ. Exp. Bot. 19: 93–97; 1978.

[1] Fisher Scientific, 2775 Pacific Dr., P.O. Box 829, Norcross, GA.
[2] Percival Manufacturing Co., Boone, IA.
[3] Baker Co., Sanford Airport, Box E, Sanford, ME.
[4] Tetko, Inc., 420 Sawmill River Rd., Elmsford, NY.

Approved by Author

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Signature Date