LIFE CYCLE OF THE MOSS, PHYSCOMITRELLA PATENS, IN CULTURE

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SUMMARY: A method employing tissue culture techniques for growth of mosses is described. This method allows for the completion of the sexual life cycle of the moss Physcomitrella patens (Hedw.) BSG under controlled conditions in a two month period. The moss system is useful for class demonstration or for research in the areas of development, genetics and plant physiology.

Key words: Moss; Physcomitrella patens; plant tissue culture.

I. INTRODUCTION

The bryophytes are good organisms for studying many types of biochemical, genetic and developmental processes. They are haploid, the cells are totipotent, they will grow on a defined medium under controlled environmental conditions, and several will complete their life cycle in culture (1-3). The experimental organism described here is the moss Physcomitrella patens (Hedw.) BSG. It was originally isolated from material collected in Gransden wood, Huntingshire, England, by H. L. K. Whitehouse. This species represents an especially promising tool in that several classes of biochemical mutants have been isolated and studied genetically (1,2,4). Protoplasts have been obtained (5-7) and used for somatic hybridization (4,6). The following procedure outlines the methods used to culture Physcomitrella patens and the conditions necessary for the completion of the life cycle. The procedure has been used successfully with two other moss species of the Funariaceae, Physcomitrium pyriforme (Hedw.) Hampe and Funaria hygrometrica (Hedw.). With minor alterations in the growth cycle, the procedure should be applicable to a wide variety of moss species.

II. MATERIALS

A. Equipment

- Constant temperature environmental chamber with fluorescent lighting, No. E-54U Percival
- Laminar flow hood, Baker Edge-Gard
- pH meter, No. 13-637-650 Fisher
- Hot plate with magnetic stirrer, No. 11-496-8
- Autoclave, No. 14-488-20, or pressure cooker
- Dissecting microscope, Stereo, AO 43RT

B. Medium and chemicals (All chemicals are from Fisher

1. Knops Stock Solution (8), Modified
   Ca(NO₃)₂·4H₂O 0.5 g/l
   KNO₃ 0.125 g/l
   MgSO₄·7H₂O 0.125 g/l
   KH₂PO₄ 0.125 g/l

2. Nitch's Minor Elements Stock Solution (8)
   H₂SO₄, sg 1.83 0.5 ml/l
   MnCl₂·4H₂O 2.5 g/l
   H₂BO₄ 2.0 g/l
   ZnSO₄·7H₂O 0.05 g/l
   CoCl₂·6H₂O 0.03 g/l
   CuCl₂·2H₂O 0.015 g/l
   NaMoO₄·2H₂O 0.025 g/l

3. Ferric Citrate Stock Solution
   Fe(Cu)H₂O·5H₂O 25.0 g/l (raise pH to get into solution)

4. Agar, SP 8.0 g/l (optional, see Procedure, A.2)

C. Glassware and other

- Petri dishes, 15 x 150 mm, plastic, No. 08-757-14
- Erlenmeyer flasks, 125 ml, No. 10-040D
- Glass funnels, No. 10-346-5A
- Coil cotton, 25 mm, No. 07-885D
- Aluminum foil, purchased locally
- Pasteur pipettes, No. 13-678-20A
- Forceps, No. 08-8803
- Dissecting needles, No. 08-960A
- Parafilm, No. 13-374-5
- Nitex cloth, 64 micron pore size, Tetko Inc.
- Test tubes, No. 14-9563
- Blender, No. 14-509-17
- Blender container, No. 14-509-18A
- Magnetic stir bar, No. 14-511-66
- Chlorox, purchased locally
- Ethanol, 95%
D. Moss cultures
Available from Dr. Karen W. Hughes.

III. PROCEDURES

A. Preparation of materials

1. Use standard sterilization techniques for all glassware, instruments used for transfers, and media.

2. Preparation of medium

a. Combine 500 ml of Knops Stock, 0.5 ml of Nitsch's Minor elements, 0.4 ml of ferric citrate solution and 500 ml of double distilled water. Bring to pH 5.5. If solid cultures are desired, heat the medium and add 8.0 g/l agar. Bring to a boil while stirring to dissolve agar. Dispense to containers and autoclave to sterilize.

b. For liquid cultures, dispense 50 ml of liquid medium (without agar) into 125 Erlenmeyer flasks. Plug the flasks with cotton, cap with aluminum foil and autoclave.

c. For the isolation of spores, dispense 10 ml of liquid medium into 25 ml test tubes. Plug the tubes with cotton, cap with aluminum foil and autoclave.

d. For solid culture, dispense 25 ml of medium containing agar into 150 ml glass petri plates, and autoclave. For plastic plates, autoclave the medium and cool to 50°C prior to dispensing to the plates.

B. Establishment of moss cultures

Initial cultures are established from spores because of the difficulty in decontaminating moss gametophytic tissues; however, once the plants are established in culture, tissue from the plants may be used for subculture as described below.

The leafy plant bears gametangia in which gametes, non-motile egg and flagellated sperm, are produced by mitosis. Fertilization is accomplished by the sperm swimming to the egg in a drop or thin film of water. The resultant zygote germinates into the sporophyte (2N) which produces the spore capsule. Spores of Physcomitrella patens are produced in a cleistogamous (non-dehiscent) capsule which is not elevated above the leafy gametophyte. Each spore is the result of meiosis; consequently, each is haploid and capable of germinating directly into a new haploid plant.

1. With the aid of a dissecting microscope, select an unopened (undehisced) capsule from a mature culture or plant or from a dried specimen.

2. Surface sterilize the capsule by dipping it in 70% ethanol to wet the surface then place it into commercial Clorox for 5 minutes.

3. Place the capsule on the inside wall of a small test tube containing 10 ml of sterile liquid medium. Break the capsule open with a dissecting needle to liberate the spore mass.

4. Replug the test tube with cotton and tip the tube to allow the liquid medium to pick up and disperse the spores. The resultant spore suspension should contain approximately 1-2 x 10^3 spores/ml of medium (3).

5. Spores may be germinated in this medium for 2-3 days or used as an inoculum immediately.

6. Liquid cultures are established by adding aliquots of the spore suspension to 50 ml of medium in Erlenmeyer flasks. Liquid cultures will grow primarily as protonema and will produce gametophytes only when the cultures are old.

7. Solid cultures are established by plating aliquots of the spore suspension onto agarized medium in petri dishes. The dishes are sealed with strips of parafilm to prevent dehydration. Solid cultures will briefly grow as protonema then will become gametophyte colonies. Each gametophyte is the product of a single protonemal cell.

8. Spores may be plated at low densities from an initial spore isolate in order to obtain gametophyte colonies with a single spore origin. Such colonies are genetically uniform. Subsequent isolations from a single gametophyte colony, either from spores or from blended plant tissue, will be genetically uniform. All of our cultures are clonal and are genetically uniform.

C. Subculture of moss

Since cells of the moss are totipotent, any portion of the green plant may be used as inocu-