Axenic Culture of the Aquatic Fern, Salvinia[1]

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Salvinia has potential as an experimental organism because of its small size, simplicity, and rapid growth. A limited amount of work has been done on reproduction and growth of *Salvinia*. Cultural studies employing nonsterile conditions have revealed information on vegetative growth (Rajan, Betteridge & Blackman, 1971), photoperiodic responses (Nakayama, 1952), and competitive growth (Chatworthy & Harper, 1962). The effect of growth regulators (Gaudet & Huang, 1967; Gaudet & Koh, 1968) and growth in a simulated natural environment (Gaudet, 1973) have been studied using aseptic culture. It is the intent of this note to report a simple, reproducible method by which *Salvinia* can be surface-sterilized and cultured aseptically.

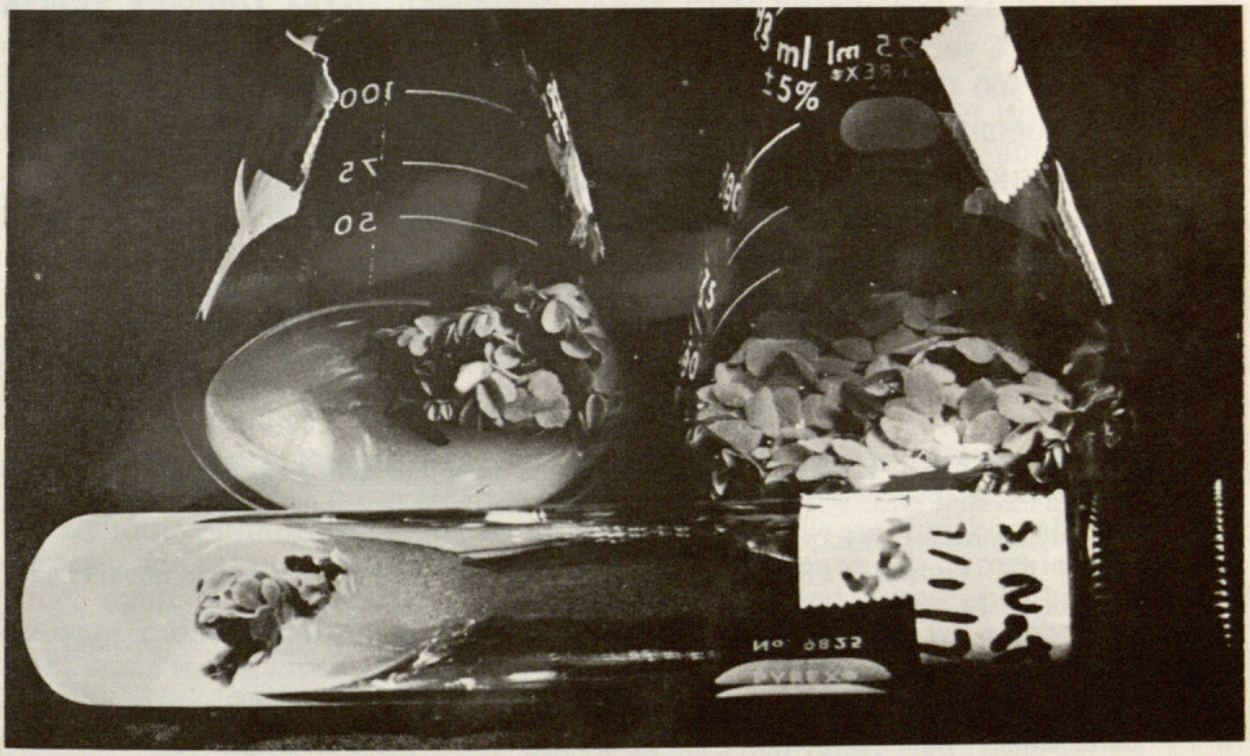

FIG. 1. Sterile stock culture of *Salvinia natans* in test tube and *Salvinia auriculata* in 125 ml Erlenmeyer flask (left) on Hutner's medium solidified with agar. Erlenmeyer flask (right) shows crowded pure culture of *S. auriculata* on liquid Hutner's medium.

The nonwetting property of the floating leaves makes sterilization of *Salvinia* difficult. Gaudet and Koh (1968) have reported a method of surface sterilizing *Salvinia* which I found difficult to reproduce. Their method was not described in sufficient detail to be reproduced and proved unreliable. The method reported here is similar to that used for surface sterilizing *Lemna* (Hillman, 1961). Plants consisting of the two youngest floating leaves, one submerged leaf, and a submerged shoot apex, which are relatively free of debris and other organisms, are

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selected under a dissecting microscope and washed three times in sterile tap water in a 250 ml Erlenmeyer flask by shaking with a test tube shaker (Super-mixer, Matheson Scientific Co., Chicago) for about one minute. Then they are shaken in a sterile aqueous solution of Tween 80 (10^{-5}, v/v) to break the surface tension of resistant fungal and bacterial spores. The plants are left in sterile tap water for 24 hours to allow resistant spores to germinate. Then they are immersed in 0.2% bleach (a 1 to 26 dilution of 5.25% commercial sodium hypochlorite bleach) until the leaves begin to turn white (ca 2–5 min). Bleach obtained at different times may be of different strengths; therefore, some trial bleaching of fronds is necessary to determine the proper length of time in the bleach. The first plants removed should be bleached only slightly, whereas the last plants removed should be bleached completely white. The bleach is removed by two rinses in sterile tap water and three rinses in sterile distilled water with brief shaking. The plants are placed singly in 60 × 15 mm plastic Petri plates containing sterile Hutner's growth medium with 1% sucrose (Hutner, 1953). Cultures placed in continuous fluorescent light (ca 300 ft-c) at about 30°C should show growth of *Salvinia* and the presence of contaminates (if any) within one week. After sterile plants have been obtained, they grow well in 125 ml Erlenmeyer flasks with 50 ml of Hutner's growth medium. In reduced continuous light (125 ft-c) and temperature (about 22°C) stock cultures can be maintained on growth media solidified with 1% agar for 6 months or longer (*Fig. 1*). *Salvinia natans* and *S. auriculata* have been obtained successfully in pure culture using this technique.

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