Axenic Culture of Hydrilla

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INTRODUCTION

Infestations of hydrilla have become increasingly troublesome in east Texas because they cause loss of useable surface area in lakes. One approach to the control of this aquatic angiosperm involves an attempt at life-cycle interruption augmented with herbicidal treatment. Specifically, prevention of the formation of subterranean vegetative buds or tubers would be important towards developing effective hydrilla management. Contaminant-free cultures are required to investigate the biochemical interactions involved in the tuberization process and the purpose of this study was to develop a procedure for obtaining axenic cultures.

MATERIALS AND METHODS

The procedure developed for obtaining axenic cultures of hydrilla utilized tubers. Each tuber was treated for 20 minutes in 1% NaOCl. The outer tissue layers were removed using sterile dissecting instruments. Growth medium was 10% Hoagland's augmented with 150 mg/l penicillin, 100 mg/l streptomycin, 25 mg/l gentamicin, and 25 mg/l mycostatin. Glucose (2%) and casein digest extract (0.5%) were added to facilitate detection of contaminants.

Sodium hypochlorite treatment, tissue removal, addition of the antibiotics to the medium, and addition of the organics to the medium were each tested for their effects on growth rate of the sprout, sprout length (yield), and success in obtaining contaminant-free cultures. A complete factorial experiment with two levels of each of six variables was performed. Four replicates of each treatment gave a total of 256 treatments.

All transfers, treatments, and dissections were performed in an ultraviolet light box (Sylvania-Germicidal G30 + 8). Each tuber was treated according to the experimental design and cultured in Petri dishes on light tables. Fluorescent lamps provided an illumination of 28 $\mu Ein \cdot m^{-2} \cdot sec^{-1}$ at a temperature of 29 C. The growth medium was 10% Hoagland's solution supplemented with 200 mg/l $NaHCO_3$.

The antibiotics penicillin, streptomycin, and gentamicin were obtained from Pfizer, Inc. The fungicide, mycostatin, was obtained from Squibb and Sons, Inc.

Data collection was done on alternate days for 21 days. Length of sprout, number of nodes, and presence of visible contamination were noted. After 21 days, a 1 ml sample from each culture was placed in a test tube containing thioglycolate broth and incubated for 72 hours to detect contamination.

Semi-logarithmic plots of length versus time were used to determine maximum growth rate of each culture. Total length after 21 days was used as final yield.

RESULTS AND DISCUSSION

Growth rates, yields, and contamination data were used as parameters in an analysis of variance program to determine main variable effects as well as two-way and three-way variable interaction effects. The main variables tested were NaOCl treatment, tissue removal, penicillin and streptomycin, gentamicin, mycostatin, and the addition of organics to the medium.

No variable significantly affected growth rate. In fact, only 15% of the entire variation in slopes could be attributed to the variables. Apparently, upon sprouting, the plant maintains a constant growth rate for a given period of time. This value and the variance of this growth rate were similar for all treatments. It is the time period of growth that is most affected by the variables. Hence, final yield was used in the analysis of variance program.

Four variables significantly affected total length of the plants. Sodium hypochlorite treatment, tissue removal, and the addition of penicillin and streptomycin all reduced yield. Only the presence of organics enhanced yield.

These results could indicate that 1) hydrilla plants utilize the casein digest extract and/or the glucose, 2) the organics stimulated bacterial growth in the contaminated cultures such that extracellular organic compounds are produced

that are required and utilized by the plants, or 3) the presence of bacteria in the contaminated systems provided a constant supply of inorganic carbon through respiration.

The effect of contamination on yield of hydrilla was examined. Presentation of the data in bar graph form supports the third possibility mentioned above (Figure 1). The difference in yields between contaminated and uncontaminated cultures is significant at the 99% level. The main influence on yield is the presence or absence of contamination. These results rule out the possibility that increased yield of hydrilla could be a result of the utilization of glucose or casein digest extract.

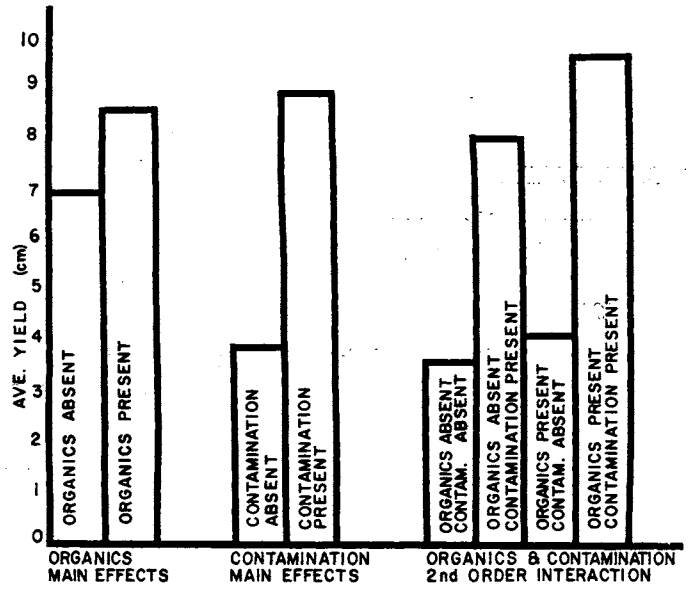

Figure 1. Average length of hydrilla stems (yield) after 21 days of growth as influenced by contamination and organics.

Four variables significantly enhance the probability of obtaining axenic cultures. The combinations of variables required for axenic cultures were found to be 1) sodium hypochlorite treatment followed by tissue removal and growth in medium augmented with penicillin and streptomycin, and 2) tissue removal followed by growth in medium containing all three antibiotics (Table 1). It appears that sodium hypochlorite treatment can be substituted for by gentamicin.

Penicillin is known to inhibit cell wall synthesis in growing cultures of gram-positive bacteria. Streptomycin affects protein synthesis in many bacteria, both gram-positive and gram-negative. Combined, these antibiotics control a large range of bacteria. However, complete antimicrobial activity cannot be obtained by these two alone. The antimicrobial spectrum for gentamicin is much greater than for streptomycin and in combinations with penicillin and streptomycin appears sufficient to control all bacteria remaining after removal of the outer layer of tissue from tubers.

ACKNOWLEDGMENTS

The authors appreciate the assistance of Dr. D. L. Sutton, University of Florida, Fort Lauderdale, in obtaining hydrilla tubers.

This investigation was supported by an Amoco Foundation Fellowship to the Senior Author and by Environmental Protection Agency Grant No. T-900966-01.

TABLE 1. COMBINATIONS OF VARIABLES NECESSARY FOR AXENIC CULTURES OF HYDRILLA FROM TUBERS.[1]

NaOCl Treatment	Tissue Removal	Penicillin & Streptomycin	Gentamicin	Mycostatin	Organics	Axenic?
+	+	+	±	±	±	Yes
+	+	−	±	±	±	No
+	−	±	±	±	±	No
−	+	+	+	±	±	Yes
−	−	±	±	±	±	No

[1] + indicates variable required for axenic cultures. − indicates variable not required for axenic cultures. ± indicates that neither the presence nor the absence of the variable affected the probability of obtaining axenic cultures.