PRODUCTION OF SOLAVETIVONE BY IMMOBILIZED CELLS
OF HYOSCYAMUS MUTICUS

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SUMMARY. The secretion of solavetivone, a phytoalexin, by the cells of Hyoscyamus muticus in response to fungal elicitation has been enhanced by gel entrapment in calcium alginate. The immobilized cells produced 53% higher product and exhibited sustained biosynthetic activity in repeated batch cycles when compared with suspended cells. Providing a non-elicited media exchange and a sufficient interval of time between repeated infection (elicitation) gave greater productivity. Apparently the cells need a period to recover from the infection.

INTRODUCTION

Immobilization of plant cells in calcium alginate has resulted in increased de novo synthesis of secondary metabolites of numerous plant cell cultures: Catharanthus roseus (Brodelius, 1983; Asada and Shuler, 1989), Coffea arabica (Haldimann and Brodelius, 1987), Lithospermum erythrorhizon (Kim and Chang, 1990), Morinda citrifolia (Brodelius et al., 1980) and Solanum surattense (Malpathak and David, 1992). The myriad of potential explanations for increased metabolite formation can be classified into several categories: Alteration of physical environment: Physical entrapment of plant cells protects cells from the fluid shear forces of culture conditions. Immobilization also results in direct cell-surface contact (which is very important for mammalian cells). Alteration of chemical environment: The matrix surrounding the cells will result in the establishment of diffusional gradients both of nutrients and waste products. This matrix could also act as a source or sink for chemical species such as toxins or calcium. Alteration of physiological environment: When cells proliferate within a matrix, they are likely to remain interconnected by plasmodesmata. These channels provide inter-cell communication and could result in a pseudo-differentiated state. Alteration of physiological condition: As a result of the alterations in physical and chemical environment, cells grown within an alginate matrix are likely to exhibit physiological changes which are independent of cell-
cell proximity. The most notable change is cell growth rate. Since growth rate is known to have a strong effect on secondary metabolism due to competition for resources, reduction in growth rate imposed by immobilization may play an important role in enhanced rates of secondary metabolite production.

The majority of the physiological effects could be eliminated if the growth phase within the matrix could be eliminated. The plant cell types which have been immobilized thus far produce phytochemicals which display predominantly growth associated production, and product formation takes place over a several week period. Sesquiterpenes are a class of phytoalexin which rapidly accumulate within 12 to 24 hours after exposure to fungal elicitors (Heinstein, 1985; Brindle et al., 1988; Dunlop and Curtis, 1991). In this study we use fungally elicited cell suspensions of Hyoscyamus muticus to examine the short-term effects of immobilization. It is shown that although no sesquiterpenes accumulate with immobilization alone, immobilization results in nearly a 2-fold enhancement in fungally elicited solavetivone accumulation within 24 hours. These results clearly demonstrate that the physical/chemical environment of immobilized cells plays an important role in the enhancement of secondary metabolite formation. Further enhancement of productivity is achieved by repeated elicitation of the immobilized cells and with an optimal period for stress recovery between successive elicitations.

MATERIALS AND METHODS

Culture: Suspension cultures of Hyoscyamus muticus were maintained by bi-weekly sub culturing on 50 ml B5 medium with 0.2 mg/l 2,4-D in 125 ml Erlenmeyer flasks. The cultures were incubated on a gyratory shaker with a 2-in stroke at 80 rpm and 25°C.

Immobilization of Cells: Two week old cell suspension cultures of H. muticus were harvested under aseptic conditions and used for immobilization. Desired amount of cells (3 g FW) in 25 ml of medium were mixed with equal amount of sterile sodium alginate (4%) and the resultant viscous suspension was extruded through a nozzle by means of a peristaltic pump into sterile calcium chloride (0.05 M). The resultant gel beads (4 mm dia) were cured for 2 hours and transferred to sterile saline (0.85%) and stored at 4°C until use. An experimentally determined water content of 96.9% was used to calculate dry cell loadings.

Preparation of Elicitor: The fungal elicitor was prepared from the soil fungus Rhizoctonia solani (Signs and Flores, 1989) grown in a 5 L Bioflow III (New Brunswick Scientific, Edison, N.J.) in SH medium at 200 rpm with Rushton impellers and no baffles. The elicitor was prepared by resuspending mycelia in Mill-Q water (0.3 g FW/L) followed by homogenization and autoclaving for 3 hours. The elicitor consisted of the hydrolyzed supernatant after centrifugation for 30 minutes at 20,000 rpm (Dunlop and Curtis, 1991).

Batch Culturing: Equal amounts of free and immobilized cells (1 g FW) were inoculated in 50 ml B5 medium with 0.2% 2-4-D in 125 ml flasks. Two ml of sterile fungal elicitor was added after inoculation. The cultures were maintained on a gyratory shaker at 25°C for 24 hours. Samples were collected after incubation and analyzed for solavetivone.

Elicitor Pre-treatment: Equal amounts of cells (3 g FW) were suspended in 50 ml medium containing 2 ml of elicitor and maintained on a shaker for different intervals of time (1 to 24 h). The elicited cells were subsequently filtered and immobilized as above. The pre-