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SOLASODINE PRODUCTION BY IMMOBILIZED CELLS AND SUSPENSION CULTURES OF SOLANUM SURATTENSE

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Summary

Immobilized cells of Solanum surattense Burm release far more solasodine into the medium than free cell suspension cultures. This enhancement is probably due to stabilization of cells after immobilization as well as the effect of growth hormones in the medium.

Introduction

Immobilization of plant cells has been reported to increase their potential for releasing secondary products into the medium as compared to freely suspended cells (Brodelius and Nilson, 1979, 1983; Majerius and Pareilleux, 1986, Lindsey and Yeoman, 1987). Solanum surattense is a fairly rich source of solasodine (Kaul and Zutshi, 1982) which is also formed in callus cultures. (Heble and Narayanswami, 1968; Khanna et al; 1976, Barnabas and David, 1986). The purpose of this study was to compare the solasodine producing potential of immobilized and free cell suspensions of S. surattense.

Materials and Methods

Cell suspension cultures : The Solanum surattense cell suspension cultures were obtained from leaf callus by placing 1-2g of callus in shake flasks containing MS medium (Murashige and Skoog, 1962) supplemented with 1 ppm kinetin and 2 ppm NAA. The cell cultures were maintained at 25^{0}C in continuous light and agitated at a speed of 70 r.p.m. Subcultures were done every 7 days by adding 10-15 ml of culture of 50 ml of fresh medium.

Immobilization of cells : Immobilization in calciumalginate was achieved by filtering through a wire mesh of area 4 mm^{2} per pixel, into a sterile flask. After allowing the cells to settle for about 30 min. the supernatant was decanted leaving behind a suspension of cells. A solution of 3% alginate prepared in MS medium was mixed with suspended cells 1:1 (V/V). The viscous suspension was allowed to drop slowly through a sterile syringe into a medium containing 50 mM $CaCl_2$. The beads so formed were left in this solution for 30 min.

and then washed with several changes of sterile distilled water. Cultures of immobilized cells were initiated by inoculating 30 beads in 25 ml of a) a hormone free medium and b) a medium containing kinetin and NAA. Both the suspension cultures and the immobilized cell cultures were maintained under identical conditions.

Analytical procedures : The percentage of viable cells was estimated from ten replicates per treatment by staining the beads with a 10% solution of 2,3,5 tetrazonium chloride (TTC) in Sorensons phosphate buffer (M/15, pH = 6.8). The viable cells stained a pink colour after incubation at 22-25^{0} C.

Suspended as well as immobilized cells were filtered, blotted dry and then weighed. The extraction of solasodine and quantitative determination was done as described by Bhatt et al., 1983.

Results and Discussion

The suspension cells have a high intracelluar accumulation of solasodine (figure 1), but the immobilized cells released much higher quantities. Jirku et al., (1981) also reported extracellular accumulation of steroidal glycoalkaloids of immobilized cells of Solanum aviculare. The exudates of Solanum surattense contained 5 times more solasodine in hormone free medium and 3 times more in hormone supplemented medium than in the medium in which cells were freely suspended after 12 days of incubation (figure 2). The decline in solasodine release in the hormone-free medium after 19 days could be due to the complete utilization of the intrinsic growth hormones. In the hormone supplemented medium there was a continued further increase even upto 27 days of incubation. This could be due to the physical and chemical gradients in the matrix of calciumalginate beads which act to stabilize the cells and provide greater drainage, resulting in an enhanced solasodine release. Such accumulation has also been observed in case of indole alkaloids (Majerius and Pariellux, 1986). It is possible that the immobilized cells become subject to a nutritional stress which in turn may be particularly useful by bringing cells to an early stationary phase causing an increase in alkaloid level (Lindsey et al., 1983).

The percentage viability obtained was 70% at the end of the 12th day in both suspended and immobilized cells, thus the viability of cultures is not affected by immobilization procedures.

It appears from the results that the total amount of glycoalkaloids present is not different, only more alkaloid is trapped within the cell matrix in suspension cultures while it continues to be released (drained) into the medium in immobilized cell cultures. This release is of advantage because the amounts of solasodine released can be concentrated by recirculation of the spent medium.

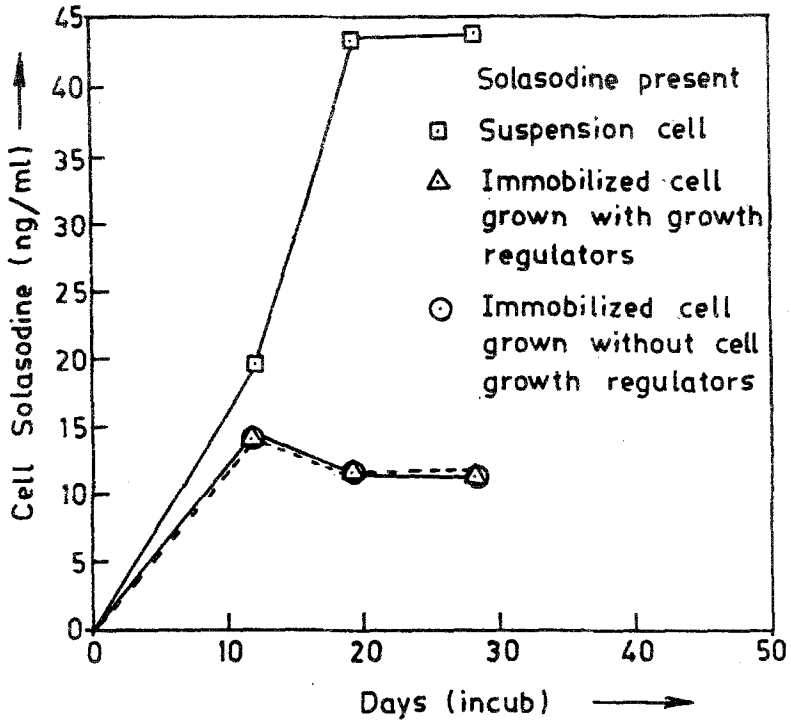

Figure 1- Solasodine content of the suspended and immobilized cell. Each point is a mean of triplicates.

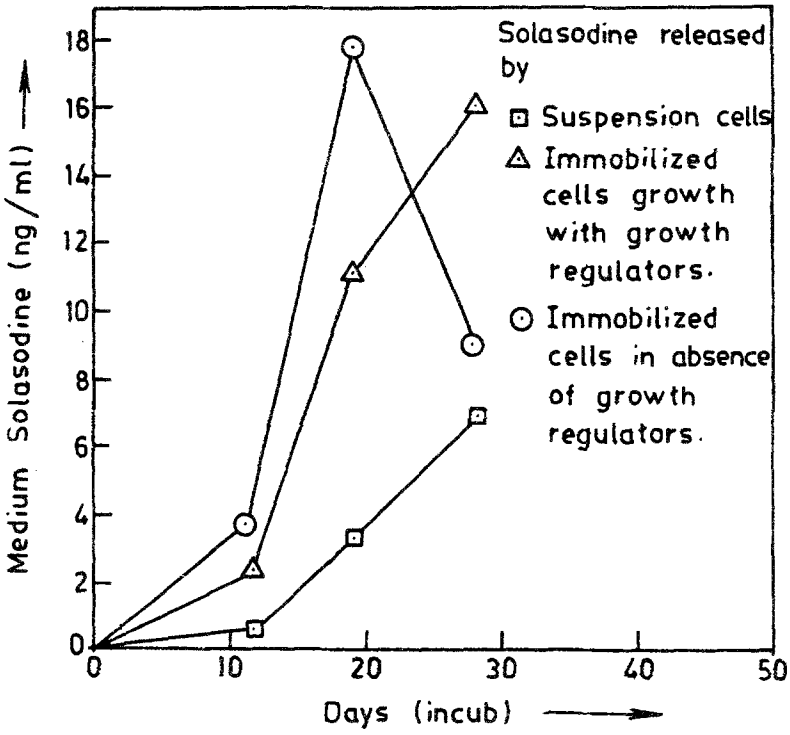

Figure 2- The release of solasodine by suspended and immobilized cells in the medium. Each point is a mean of triplicates.

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