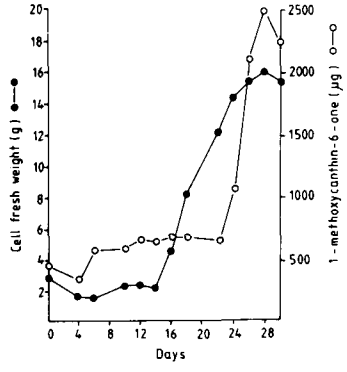
GROWTH AND ALKALOID ACCUMULATION IN CELL SUSPENSION CULTURES OF AILANTHUS ALTISSIMA

L.A. Anderson, J.D. Phillipson, M.F. Roberts, Department of Pharmacognosy, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX

An understanding of the relationship between the growth cycle and the accumulation of secondary metabolites is essential for optimum exploitation of plant cell cultures as sources of pharmaceuticals, such as plant alkaloids. The relatively few studies which have been undertaken indicate, in general, that maximum accumulation of secondary products occurs when the growth rate decreases (Yeoman et al 1980). However, in some instances, growth and alkaloid production parallel each other (Hinz and Zenk, 1981) whilst in others maximum accumulation of alkaloids occurs early in the linear growth phase and then the levels drop sharply as the alkaloids are further metabolised (Berlin et al, 1983).

High alkaloid yielding cultures of Ailanthus altissima (Mill.) Swingle, have been maintained in our laboratories for 3 years and are being used as a model system to study alkaloid production in cell cultures (Anderson et al, 1983). A time-course experiment has been carried out using 6 month old suspension cultures in order to establish the relationship between growth and alkaloid production. Homogenous suspensions, consisting of fine cells and small aggregates, were filtered aseptically and the cells distributed into flasks containing 40 ml medium, giving an initial inoculum of 3g fresh weight per flask. Flasks were harvested on alternate days throughout the growth cycle, the cells were filtered from the medium and their fresh weight determined. Alkaloids were extracted from the cells with methanol, further purified using Extrelut columns and analysed quantitatively by HPLC (Anderson et al, 1983). The growth characteristics and the pattern of alkaloid accumulation for A. altissima suspension cultures are shown in figure 1. The growth cycle is characterised by a significantly long lag phase (14 days) and a short exponential growth phase (4 days). There follows a long linear growth phase of 10 days before the cells enter the stationary phase. From day 14 until day 22 the total alkaloid content remains the same but the yield of alkaloid per gram fresh weight of cells falls dramatically from 285 to 53 µg/g f.wt., as the growth increases rapidly. The short period from day 22 to day 28 is characterised by rapid production of the major alkaloid, 1-methoxy-canthin-6-one. During this stage, the growth rate is decelerating at the end of the growth phase and there appears to be a switch away from primary towards secondary metabolism. The alkaloid levels reach a maximum at day 28 as the cells enter the stationary phase. Harvesting the batch cultures at this stage would ensure optimum yield of 64mgl^{-1} per cycle of 1-methoxycanthin-6-one from these cultures. The yield of alkaloid per g f.wt. of the final biomass is equivalent to that of the initial inoculum and high alkaloid levels are maintained in these cultures.

Time course of the fresh weight and 1-methoxycanthin-6-one accumulation in A altissima suspension cultures



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Anderson, L.A. et al (1983) J. Nat. Prod. 46: 374-378
Berlin, J. et al (1983) Z. Naturforsch. 38: 346-352
Yeoman, M.M. et al (1980) Plant Cell Cultures: Sala, F. et al eds. Elsevier/North Holland Biomedical Press, Amsterdam, pp. 327-343