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Immobilized Plant Cells

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Abstract

Plant cells have been immobilized in alginate, where they have been shown to retain their biological activity. Such systems can be utilized for bioconversions.

Index Entries:Immobilized plant cells; plant cells, immobilized; cells, immobilized plant; *Catharanthus roseus*, immobilized cells from; ajmalicine, from immobilized plant cells; *Digitalis lanata*, immobilized cells from.

Introduction

Today a large number of compounds are produced from higher plants, e.g., pharmaceuticals, food additives, and oils. The production of many important pharmaceuticals is thus dependent upon raw materials extracted from tropical plants in many cases, and is therefore also dependent upon distant producers. The risk of supply shortage has focused recent research on the development of alternative production methods for natural products. There is also at present a shortage of certain natural products, which could, however, be covered if such alternative production methods were available.

Moreover, the substances utilized for the production of pharmaceuticals and as food additives are mostly secondary metabolites. For this reason they are often present in very low concentrations in plant tissue. As a consequence, large amounts of plant material are required for the isolation of a given substance in substantial amounts. Nevertheless, a great many pharmaceuticals are at present produced from higher plants, such as steroids (from diosgenin), tropane alkaloids, such as atropine, hyoscyamine, and scopolamine, indole alkaloids, such as ajmaline, reserpine, vincristine, and vinblastine, heart glycosides, such as digoxin and digitoxin, morphine, codeine, and many others.

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In recent years there have indeed been increasing difficulties in securing an ample supply of some of these pharmaceutical substances because of a drastic decrease in plant resources owing to disturbance of the natural environment, ruthless exploitation, increasing labor cost, and technical and/or economic difficulties in culturing wild plants. Plant tissue cultures appear to be one possible alternative method for the production of natural compounds of economical value.

Both callus and suspension cultures of the plant in question might be used. The low productivity of natural products in such cell cultures is, however, a recognized problem and many attempts are being made to manipulate the overall metabolism of the cells in order to increase the total yield of the product in question. For some time, we have been investigating whether plant cells obtained from tissue culture can readily be "manipulated" in an immobilized state to produce increased quantities of secondary metabolites. We have demonstrated that immobilization of plant cells offers some distinct advantages (*1*). In Fig. 1, some alternative approaches to the preparation and immobilization of plant cells are shown.

We have found that cell proliferation is very efficient within the beads, leading to actual "cracking" of the latter after incubation for some time in a complete medium. Omission of growth hormone in the medium, however, prevented cell division to such an extent. An important observation was made under such hormone-free conditions: namely, that one class of secondary products studied, anthraquinones from *Morinda* cells, accumulated in the immobilized cells with a factor of ten compared to cells in free suspension (*2*). If this phenomenon turns out to be of general validity (other systems are at present under investigation), then this should have great importance for the production of secondary metabolites. Furthermore, we have observed that the overall "life length" of cells is increased in the immobilized protected form, which could lead to higher productivity of secondary products.

In the example described above, the product, i.e., the anthraquinones, was synthesized *de novo* from sucrose as carbon source. Plant tissue cultures, however, offer a general advantage in that suitable precursors can be added to the growth medium. The precursors are relatively efficiently utilized for synthesis of secondary products. Thus, in a model study we have shown that immobilized cells of *Catharanthus roseus* possess the ability to synthesize the indole alkaloid ajmalicine from the distant predcursors tryptamine and secologanine in high yields (*1*, *2*). To carry out a similar synthesis with an intact plant practically is more difficult and the yields are much lower.

Another type of reaction that can with advantage be carried out in tissue cultures is simple biotransformation. In model studies with immobilized cells of *Digitalis lanata*, the 12-β-hydroxylation of digitoxin to digoxin has been efficiently carried out (*1*, *2*).

The permeability of the membranes of immobilized cells can also be conveniently changed, as shown by us recently (*3*), to allow leakage of products normally stored within the cells. By the same token, required precursors can more easily be made to enter the cells.

In case it is impossible to cultivate cells in vitro, an alternative procedure might be applied: namely, preparation of protoplasts from the intact plant with subse-

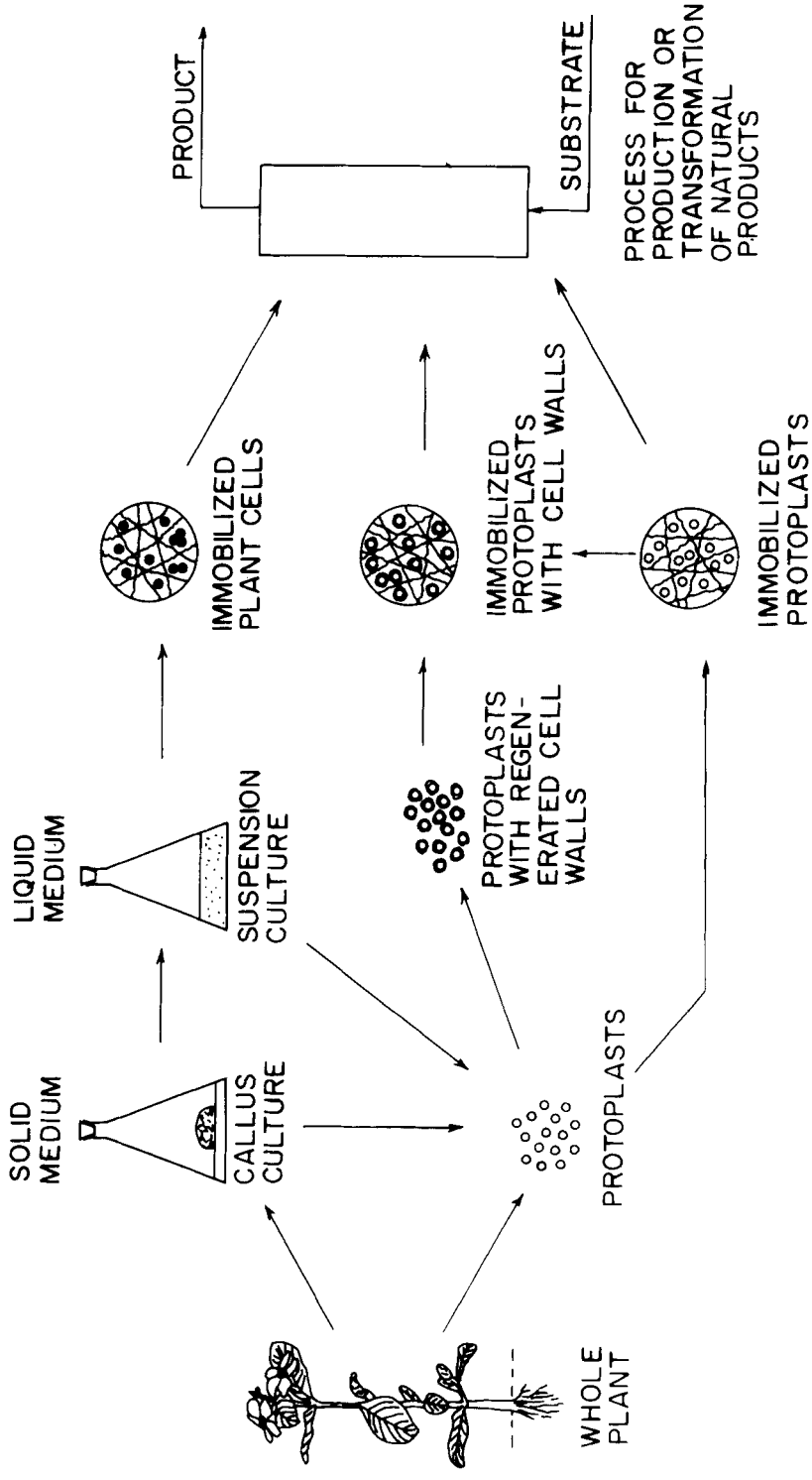

Fig. 1. Alternative approaches to preparation and immobilization of cells from higher plants.

quent immobilization (see Fig. 1). We have observed that such fragile cells are considerably more stable in an immobilized state and therefore easier to handle. Furthermore, the cells can be expected to stay alive a considerable time in the immobilized state and thereby are capable of producing secondary metabolites. In addition to this, the administration of precursors can be improved considerably as compared to the intact plant for biosynthetic studies.

In all our initial studies the plant cells have been immobilized by entrapment in alginate, but recently we have also investigated other matrices, such as agar, agarose, carrageenan, and gelatin (*4*).

In conclusion, the use of immobilized plant cells appears to have added a new useful technique to plant tissue culture with the emphasis on secondary product formation.

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