INFLUENCE OF EXTERNAL pH ON ALKALOID PRODUCTION AND EXCRETION BY CATHARANTHUS ROSEUS RESTING CELL SUSPENSIONS

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INTRODUCTION

Catharanthus roseus (L.) G. Don, cell line C20, cultivated in a batch culture system with a modified Gamborg's B5 medium (Gamborg et al., 1968) deprived in auxin (2,4-dichlorophenoxy-acetic acid) and enriched in mannitol (200 mM) were maintained in survival conditions during 10 days after the growth phase (Ambid et al., 1982). The production of two alkaloids : ajmalicine and serpentine, was then largely emphazised (Roustan et al., 1982) and these compounds were essentially located within the cells.

In the present study, the same medium was used in a closed continuous culture system, and the alkaloid production and excretion were then examined, in relation with the external culture pH.

MATERIAL AND METHODS

The culture was achieved in a bioreactor (2 liters capacity) by inoculating 250 ml of a 7 days old batch culture grown in standard B5 medium (4.5 μM 2,4-D, 0.28 μM kinetin) in the vessel containing modified B5 medium at pH 5.5.

Cells were mechanically stirred (180 rpm) and continuously aerated (0.2 v.v.m. at the end of the growth phase); the culture was regularly supplied (0.8 ml/h) with B5 modified medium at pH 5.5 (or pH 4.3 for the experiment at acidic pH values).

Cells were maintained in the reactor and separated from the effluent by a decantation system.

Measurements of suspension growth and alkaloids extraction were carried out according to the procedure previously described (Ambid et al., 1982). For each experiment, alkaloids content was measured in the cells (internal production), in the reactor medium, and in the effluent (external production) using HPLC technique (Roustan et al., 1982); it was expressed in $\mu g\ 10^{-6}$ cells.

RESULTS

Growth Characteristics in the Culture System

In this system, where medium is continuously supplied, we observed a growth phase of 10 days (with maximal growth rate : 0.158 j^{-1}) followed by a stationary phase of 20 days characterized by a constant cell number (Fig. 1). The rate of cellular mortality increased slowly, reaching 20% after 27 days of culture. In the reactor, the pH of the medium stayed near 6.0 value when the resting cells suspension was obtained.

Figure 1

Growth characteristics of a Catharanthus roseus cells suspension cultivated in a bioreactor with a renewed medium deprived in 2,4-D and enriched in mannitol

Production and Distribution of the Alkaloids

The production of the two alkaloids was important only during the stationary phase, after 10 days of growth. At the end of the culture, this production reached respectively 0.3 and 0.7 μg 10^{-6} cells for serpentine and ajmalicine (Fig. 2-A and 2-B), with a constant amount for the ajmalicine.

These two compounds were present within the cells and, also, in the culture medium. Serpentine was largely located in the cells (Fig. 2-A) while the external amount of ajmalicine was important (Fig. 2-B).

If we examined the distribution between cells and medium, calculating the ratio : external amount on total amount of each alkaloid (Table 1), we noticed that these ratios were different for the two compounds, and relatively constant for each of them : 30% of the total serpentine and about 45% of ajmalicine were present in the medium.

Acidification of the Renewed Medium

Eight days after the beginning of the stationary phase, when the medium of the resting cells suspension was continuously supplied with the acidic medium (pH 4.3), no alteration was observed in the parameters of growth, but the distri-

Figure 2

Production and distribution of serpentine (A) and ajmalicine (B) between cells and medium for Catharanthus roseus resting-cells suspension

Table 1

Evolution of the Ratio : External Level of Alkaloid/Total Level during the Stationary Phase of Catharanthus roseus Cell Culture (in per-cent)

Alkaloid	Days of culture					
	10	13	18	21	24	28
Serpentine	50	43	32	30	28	30
Ajmalicine	63	47	48	46	46	43

bution and the production of the two major alkaloids were largely modified.

In the first 6 days of renewal at pH 4.3, the pH in the reactor was slowly acidified (Fig. 3-A). The total production of serpentine decreased (65% of the compound disappeared in the first 3 days). Its internal level decreased in an important way, but the external one remained constant, showing that there were poor production and no excretion during this period (Fig. 3-B). The total production of ajmalicine also decreased and it could be noticed that 85% of this alkaloid was found in the external medium because of the increase of its excretion (Fig. 3-C).

After 6 days of renewal at pH 4.3, the pH of the reactor rised (autoregulation of the pH by the cells). This rise was accompanied by a new synthesis of serpentine and ajmalicine (Fig. 3-B and 3-C). Serpentine was largely located in the cells, while ajmalicine was in majority present in the medium : respectively 30% and 64% of the total production of serpentine and ajmalicine were extracellular.

Figure 3

Influence of the acidification of the renewed medium on the pH in the reactor (A), on the production and distribution of serpentine (B) and ajmalicine (C)

DISCUSSION

Maintaining a Catharanthus roseus cells suspension in survival conditions allowed us to increase the production of the two major alkaloids : serpentine and ajmalicine, as well in batch conditions as in a continuous culture system. The latter allowed the continuous excretion, then the collection, of these compounds in the medium.

The constant supply of the renewed medium (pH 5.5) maintained the pH of the cell suspension about 6.0 during the stationary phase, while in batch conditions, it was shown to be subject to variations (slight alkalinisation) (Roustan et al., 1982). Continuous culture system allowed to control modifications on the pH in the reactor and, by the way, to modified considerably the distribution and the rate of production of the two alkaloids. These compounds are weakly basic and some models of transport mechanisms across cell membranes have been proposed : diffusion and accumulation in the vacuoles according to the ion-trapping model (Renaudin et al., 1982) or active and specific transport (Deus-Neumann et al., 1986).

Nevertheless, whatever the transport and accumulation mechanisms involved, non-growing cells suspensions obtained in a continuous culture system allowed important excretion of the alkaloids. This system could be an attractive biotechnological process for producing and collecting secondary metabolites of medicinal interest.

REFERENCES

Ambid, C., Roustan, J. P., Nef, C., and Fallot, J., 1982, Influence of 2,4-dichlorophenoxyacetic on the production of ajmalicine and serpentine by cell suspension cultures of Catharanthus roseus, in "Proceedings of the Fifth International Congress of Plant Tissue and Cell Culture",

Deus-Neumann, B., and Zenk, M. H., 1986, Accumulation of alkaloids in plant vacuoles does not involve an ion-trap mechanism, Planta, 167:44.

Gamborg, O. L., Miller, R. A., and Ojima, K., 1968, Nutrient requirement of suspension cultures of soybean root cells, Exp. Cell Res., 50:151.

Renaudin, J. P., and Guern, J., 1982, Compartmentation mechanisms of indole alkaloids in cell suspension cultures of Catharanthus roseus (L.) G. Don, Physiol. Vég., 20:533.

Roustan, J. P., Ambid, C., and Fallot, J., 1982, Influence de l'acide 2,4-dichlorophénoxyacétique sur l'accumulation de certains alcaloides indoliques dans les cellules quiescentes de Catharanthus roseus cultivées in vitro, Physiol. Vég., 20:523.

Nef Claudine, Ambid C., Fallot J. (1986)

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