The Effect of DMSO on Excretion of Proteins and Phenolic Substances by *Nicotiana tabacum* Cells into Culture Medium

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Abstract. Supplement of liquid culture medium with 5 % (v/v) of dimethylsulfoxide (DMSO) for two subcultivation intervals increased permeability of cell suspension culture of *Nicotiana tabacum* L. This effect resulted in reduction of fresh mass yield, increase of relative protein content and release of protein into medium during the first subcultivation. Permeabilized cells were further cultivated either in DMSO-free or DMSO-containing medium. Recovery of cells occurred in the former medium characterized by an increase in fresh mass and changes in content and excretion of proteins similar to that found during the first subcultivation in presence of DMSO. Cells cultured for the two subcultivation intervals in DMSO-containing medium undergo a physiological stress and gradually die out. It is evident that DMSO cannot be used as permeabilization agent in long term experiments.

Exploitation of biosynthetic abilities of plant cell suspension cultures is dependent on the experimental enhancement of exchange of substances between cell and culture media. Different methods can be used for permeabilization of plasmatic cell membranes (see Felix 1982 for review) which simultaneously also affect cell viability. DMSO used in 5—10 % (v/v) concentration selectively increases permeability of plasmalemma not affecting tonoplast (Delmer 1979). Careful application of this solvent increases uptake of nutrients from the medium (Fritsch and Grisebach 1975) affecting in this way cell metabolism. These properties of DMSO were also used to advantage in studies and estimation of activities of some enzymes *in vivo* (Miura and Mills 1971).

We report here the effect of DMSO applied in two subsequent cultivation intervals on excretion of phenolic substances and intra- and extracellular protein concentration in tobacco cell suspension culture.

MATERIALS AND METHODS

Stationary cell suspension culture of *Nicotiana tabacum* VBI-O was cultivated in modified medium of Heller (Opatrný and Opatrná 1976) at 27 °C. Liquid medium was supplemented with 5 % DMSO (v/v) on the

Received December 28, 1987; accepted February 10, 1988
third day of cultivation. Cells for estimation of protein and analysis of phenolic substances were collected on 5, 7, 10 and 15 day of the first subcultivation. Then cells grown in the presence of DMSO were transferred into fresh DMSO-containing (5 % v/v) or DMSO-free medium and cultured for other 13 days. Samples of cells for analysis were collected on 3, 6, 9 and 13 day of cultivation.

Phenolic substances in medium were determined after removal of protein. Solution was hydrolyzed with 2 M NaOH under nitrogen at room temperature for 4 h. Hydrolysate was acidified with 6 M HCl to pH 2 and extracted three times with the equal volumes of diethylether. Eluate was evaporated and dry residue was dissolved in 2 ml 40 % methanol. Total phenolics were estimated by Folin-Denis reagent (ZAPROMETOV 1971) using gallic acid as a standard.

Intracellular proteins were estimated in cell suspension homogenate according to LOWRY et al. (1951). Extracellular proteins were precipitated with 80 % ammonium sulphate at 0 °C for 24 h. Precipitate was collected by centrifugation, dissolved in 0.15 M phosphate buffer, pH 6.5, and protein was estimated using the same method as above.

Uptake of oxygen was measured using the oxygen electrode according to Clark in 0.15 M phosphate buffer, pH 6.5, at 27 °C.

Results are mean values of 3 repeated analyses, deviations of which did not exceed 10 %.

RESULTS AND DISCUSSION

Testing effect of different concentrations of DMSO on metabolic activity of cultured tobacco cells, it has been found that exposition of cells to 5 % DMSO for 48 h reduced dry mass yield by 13 % and rate of respiration by 22 %. Simultaneously, the amount of protein released into medium was increased by 23 % and that of phenolic substances by 131 % (Table 1). This DMSO concentration was chosen for further experiments in which dynamics of production of phenolic compounds was investigated. Inhibitory effect of DMSO on cell growth expressed as dry mass yield is evident from Fig. 1. On the other hand, the relative content of phenolics in medium of permeabilized culture was significantly increased. A decline in phenolics content in medium on the 7th day of cultivation reflects the decline of their relative content in cells during the intensive cell division when most of

<table>
<thead>
<tr>
<th>Variant</th>
<th>Fresh mass [g/flask]</th>
<th>Respiration [% of control]</th>
<th>Proteins [mg g⁻¹ (fr.m)]</th>
<th>Phenolics [µg g⁻¹ (fr.m)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.43</td>
<td>100</td>
<td>0.0634</td>
<td>3.07</td>
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<tr>
<td>0.5 % DMSO</td>
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<tr>
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<td>0.0746</td>
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<tr>
<td>5.0 % DMSO</td>
<td>1.25</td>
<td>78</td>
<td>0.0778</td>
<td>7.10</td>
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