THE INFLUENCE OF MITOMYCIN C ON THE INDUCTION OF CROWN-GALL TUMORS

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1. Introduction

The transformation of normal plant cells into tumor cells by the crown-gall organism *Agrobacterium tumefaciens* (Smith and Townsend) Conn. has been studied along different lines. One important route concerned the question whether it would be possible to induce crown-gall tumors *in vivo* by sterile bacterial fractions. However, neither sterile cell-free extracts, nor purified bacterial fractions (DNA, RNA or protein) proved to be active in this respect.

On *Datura stramonium L.* only a temporary stimulation was observed by Manigault and Stoll [1,2] using Seitz-filter sterilized solutions.

Our experiments [3] revealed that the sterilization of the DNA-, RNA- and protein solutions by filtration with Seitz EKS-I filters, prior to inoculation on wounded *Kalachoë daigremontiana* (Hamet and Perrier) resulted in adsorption of the macromolecules onto the filters, as was shown by a comparison of the ultraviolet absorption spectra before and after filtration. Only a slight adsorption was observed, if membrane filters MF 30 (Membranfilter Gesellschaft, Göttingen) were used.

In testing the tumor inducing capacity of these latter filtrates only completely negative results were obtained. As moreover heat-treated (at least 30 min at 50°C) non-viable bacteria proved unable to induce tumors (cf. also Lippincott and Lippincott [4]) it seems that the induction of crown-gall on the plant is dependent on the presence of viable *Agrobacterium tumefaciens* cells.

It is interesting in this respect that recently Kovoor [5] with *in vitro* experiments (tissue cultures) succeeded in transforming normal plant tissue into auxin-prototrophic tissue by means of large amounts of *Agrobacterium tumefaciens* DNA.

To investigate the nature of the transformation process we tried [3] to influence this in an indirect way by means of several antibiotics and antimetabolites, known to interfere with nucleic acid or protein metabolism. Of these mitomycin C gave the most interesting results, as this antibiotic, in a low concentration, suppressed tumor formation without macroscopically observable phytotoxic effects when applied on *Kalanchoë daigremontiana in vivo.*

2. Results and discussion

First we studied the effect of mitomycin C on the growth of *Agrobacterium tumefaciens* in a liquid synthetic medium, the antibiotic being added either in the lag phase or in the log phase of the culture (table 1).

In the log phase growth was inhibited to 95% at a mitomycin C concentration of 1.5×10^{-5} M. Total inhibition of growth was observed if the antibiotic was added in the beginning of the lag phase at a concentration of 3.0×10^{-6} M.

After incubation for 18 hours in the presence of 3×10^{-6} M mitomycin C, added in the lag phase, the crown-gall bacteria were separated from the medium by centrifugation and washed twice with sterile 0.9% NaCl. Renewed growth was observed on a solid mitomycin-free peptone agar medium. Inoculation of wounded *Kalanchoë daigremontiana* with such mitomycin C treated bacteria resulted in the formation of

Table 1
Growth inhibition of *A. tumefaciens* by mitomycin C.

mitomycin C concentration (M)	percentage inhibition after addition	
	in lag phase	in log phase
1.5×10^{-5}	–	95
1.2×10^{-5}	100	–
3.0×10^{-6}	100	79
1.2×10^{-6}	95	–
3.0×10^{-7}	68	44
1.5×10^{-7}	65	–
3.0×10^{-8}	26	–

crown-gall tumors. So one can conclude that, although mitomycin C inhibits the growth of the bacterial culture *in vitro*, at the above mentioned concentration it does not cause a loss of viability nor of the tumor inducing capacity of the micro-organisms. Daily treatment of wounded plants, inoculated with these washed bacteria, with 0.05 ml of 3×10^{-6} M mitomycin C during the transformation period, caused a complete inhibition of tumor formation, however, while no necrosis in the wound area was observed (fig. 1).

The inhibition was partial only when mitomycin C was applied three days after wounding and inoculation, for a period of five days. Whether this decrease of the inhibitory effect depends on a time-dependent change in membrane permeability, possibly connected with an already advanced wound healing, and thus preventing the antibiotic from entering the cells, has to be further analyzed.

From these data it is clear that mitomycin C, in the concentration range used, interferes with the tumor induction if it is added during the period in which the plant cells in the wound area are susceptible to transformation. In this connection it should be noted that recently Heberlein and Lippincott [6] found a stimulation of tumor formation by *A. tumefaciens* (pinto bean leaf test) by very low concentrations of mitomycin C (1.5×10^{-8} M). In their case concentrations in the range we used also proved to counteract tumor initiation.

The results point to a role of DNA-metabolism during the transformation period. In the meantime further, more direct evidence in this sense was obtained in recent experiments in our laboratory [7], by which it was shown that RNA synthesized *in vitro* on an *A. tumefaciens* DNA template hybridizes to a higher extent with DNA from crown-gall tissue than with that from normal host tissue. This indicates that genetic material from the inducing bacteria is present in the tumor cell. A detailed analysis of its possible correlation with the transformation to the tumorous state is in progress.

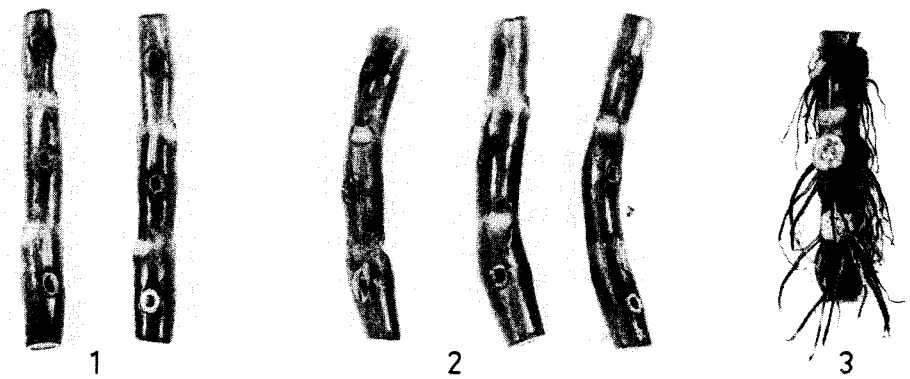

Fig. 1. Inhibition of tumor formation on the stem of *Kalanchoë daigremontiana* by mitomycin C (3×10^{-6} M). (1) Wounding and mitomycin treatment (no symptoms of phytotoxicity). (2) Wounding, inoculation with *A. tumefaciens* (strain A_6) and mitomycin treatment. (3) Control, wounding and inoculation with *A. tumefaciens* (strain A_6).

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