

## DETERMINATION OF MYCROPLASMIC CONTAMINATION BY RADIOMETRIC METHOD

**Khashimova Z.S.**

*Institute of Bioorganic Chemistry, Tashkent, Uzbekistan*



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Some species of mycoplasmas are pathogenic for man, animals and plants. The problem of mycoplasmic contamination of cultivated cells used in biotechnology for producing of monoclonal antibodies and vaccines is very actual. The plasmatic membrane of mycoplasmas, which does not differ on its structural organization from a membrane of eucariotic cells, takes part in formation of strong surface antigens. Taking into account this fact, it has been investigated mycoplasmic contamination of several kinds of cotton seeds, myelome cells XAg8.653, and hybridoma cells producing monoclonal antibodies to cotton membrane proteins. Mycoplasmic contamination was determined by hybridization of DNA of investigated objects with recombinant plasmids containing mycoplasmas DNA fragments.

DNA was isolated from 5 kinds of cotton seedling, cellular cultures and hybridomas according to standard protocols. DNA was denaturated, neutralized and a reaction mix was transferred to nitrocellulose filter. Than filters were hybridized with universal plasmid sounds namely pMg16 and pAl32, which contained the mycoplasma genomic fragment. These sounds were constructed in the Institute of Cytology of Russian Academy of Science by Borhsenius S.N. at al. [1] and kindly given us for our work. pMg16 and pAl32 were preliminary marked by  $^{32}\text{P}$  – ATP (nick – translation). Hybridization was carried out within night. Filters were washed and placed for radioautography at  $-70^{\circ}\text{C}$  for 24h. Sensitivity of  $^{32}\text{P}$  – labeled probes in reaction was 1ng of mycoplasmas DNA.

It was shown, that only one kind of cotton seeds was not contaminated by mycoplasmas, and hybridoma and myeloma cells was not contaminated. Thus, the presence of mycoplasmas in different objects may be determined using DNA hybridization.

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### Reference:

1. Borhsenius S.N. et al., *Biotechnology*, 1987, т.2, №64, p. 775.



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## T-STIMULATOR EFFECT ON COTTON PROTEIN COMPOSITION AND SYNTHESIS IN SALINIZATION STRESS

**Ibragimova E.A., Nazirova E.R., Samarkhodjaeva N.R., Nalbandyan A.A., Babaev T.A.**

*Institute of Biochemistry, Tashkent, Uzbekistan*

T-stimulator was established to possess a wide spectrum of physiological effects, to enhance plant adaptation to thermal stress and to increase plant resistance to pathogens. Plant

adaptation to unfavorable conditions manifests in changes in many links of metabolism, that of proteins included.

We studied effect of cottonseed treatment with T-stimulator on composition and synthesis of plasma membrane proteins upon chloride salinization by means of the radioisotope method. Electrophoretic fractionation of cottonseed plasma membrane proteins showed absence of more than 40 polypeptides with molecular mass from 10 to more than 100 kDa in the cotton root membranes. Major fractions-polypeptides with molecular mass of 61, 53, 46, 25, 21, 20 and 18 kDa constitute about 50% of the total polypeptide composition.

The salinization significantly affects the total membrane protein output, proportion of some polypeptides and their synthesis rate. Analysis of phoreogram radioautographs showed that 2-hour exposition of cotton roots to  $^{35}\text{S}$  methionine suppresses synthesis of major polypeptides with molecular mass of 63, 61 and 53 kDa, that of low molecular polypeptides (46, 20, 18kDa) increasing. Changes in the proportion of major polypeptides in cotton plasma membranes, reduction in rate of biosynthesis of high molecular fractions with the general suppression of label inclusion in the membrane fraction are the evidence for a disturbance in biosynthesis of some membrane proteins in cotton tissue cells upon salinization.

The inhibiting effect of salinization on the protein-synthesizing system was observed in plants treated with T-stimulator, but the rate of synthesis in plasma membranes of the treated plants was found significantly higher. The activation of some plasma membrane proteins under T-stimulator effect suggests an association with the increase in adaptation of the treated plants to the disturbing effect of salinization.



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## **VACCINES FOR VETERINARY, MADE WITH THE HELP OF RADIATIVE TECHNIQUE**

**Bulkhanov R.U., Butaev M.K., Mirsaev B.Sh., Ryasnaynskiy I.V., Yuldashev R.Yu.**  
*Scientific-Research Institute of Veterinary, Samarkand, Uzbekistan*

In applied radiology scientists usually use stimulating, mutagenic and inactivating effects of gamma-radiation.

In this report there are the results of gamma-radiation inactivating effect for radiovaccine making biotechnology development for veterinary. Inactivation with gamma-irradiation gives us opportunity to make highly immunogenic vaccines, which cause minimum damage of antigenic contaminants structure.

With the help of radiative biotechnology we can produce highly effective monoassociated and polyvalented radiovaccines against the most wide-spread infections disease in agricultural animals, young animals in particular (calves, lambs, sucking-pigs). These diseases include such infection diseases as colibacterioses, salmonellosis, pasterellosis, which cause much