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BIOCHEMISTRY, BIOPHYSICS AND MOLECULAR BIOLOGY

Using the Omega Leader Sequence of Tobacco Mosaic Virus to Transform Tomato Fruits with the Papillomavirus *hpv16* L1 Gene to Enhance Production of the Antigenic Protein HPV16 L11

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Abstract—To enhance translation of the L1 protein antigen of the oncogenic human papillomavirus type HPV16 L1, the Ω sequence of the 5'-untranslated region of tobacco mosaic virus was inserted into a genetic construct as an enhancer. To transform plants, *A. tumefaciens* EHA105 cells were transfected with this construct. After the genetic transformation, the HPV16 L1 protein antigen was detected in tomato fruit in amounts of 287–2330 ng per 1 mg total soluble protein, which significantly exceeds the amount of the protein antigen obtained in our previous studies without using the omega leader sequence.

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In the last decade, of serious concern is the increase in the infection of the population of the Earth with papillomaviruses (including the highly oncogenic HPV16 and HPV18, which cause cervical cancer), which has increased by one order of magnitude during this period [1]. The conventional preventive vaccines do not always provide 100% protection against papillomaviruses and are not free of side effects [2, 3]. Therefore, it is necessary to continue the development of a new generation of vaccines that are highly effective and safe at the same time. These conditions, in particular, are met in the vaccines based on transgenic plants, whose positive qualities have been repeatedly demonstrated [2]. In developing such vaccines, it is important to increase the content of the protein antigen in the vaccine material.

In this study, we propose a variant of creating a plant production system for the synthesis of the protein antigen of the highly oncogenic HPV type HPV16 L1 under the control of the p35S promoter of cauliflower mosaic virus and the omega leader sequence (translational enhancer) of the 5'-untranslated region of tobacco mosaic virus (TMV 5'-UTR Ω). The use of the omega leader sequence of the 5'-UTR of the TMV genome led to a significant increase in the production of the HPV16 L1 protein antigen in tomato fruit transformed with a suspension of *Agrobacterium tumefaciens* EHA105 with pBINHPV16 L1 carrying the TMV 5'-UTR Ω.

The TMV 5'-UTR Ω sequence comprises 68 bp: GUAUUUUUACAACAAUUACCAACAACAA-CAAACAACAAASAACAUUA CAAUUACUAU-UUACAAUUACA [4]. The TMV omega leader is a highly effective translational enhancer due to its unique structure, which is characterized by the following features: (1) its core part consists of ten repeats of cytidyl and adenyl CAA residues, constituting approximately half of the entire leader sequence; (2) the repeating CAA residues form a stable triple helix differing from the canonical Watson and Crick pairing, because it almost does not contain guanine residues and C : A and A : A bases in it are bound by a single hydrogen bond; (3) the leader sequence via the triple helix and AU-rich 3'-terminal region can bind to 80S and 40S ribosomes and enhance translation. The TMV omega leader can enhance translation from homologous or heterologous RNAs in different types of cells (including animal and plant cells) as well as in cell-free systems [4].

The aim of this work was to create a promising plant expression system producing the antigenic coat protein of the most highly oncogenic HPV type HPV16 L1 by genetic transformation with a suspension of *Agrobacterium* with the pBINHPV16 L1 TMV 5'-UTR Ω plasmid.

The genetic construct pBINHPV16 L1 carrying the TMV 5'-UTR Ω (Fig. 1) was synthesized by the Genscript company (United States).

In this genetic construct, the hpv16 L1 target gene was inserted downstream of the CaMV p35S promoter. The leader sequence of tobacco mosaic virus TMV 5'-UTR Ω, which is a translational enhancer, was

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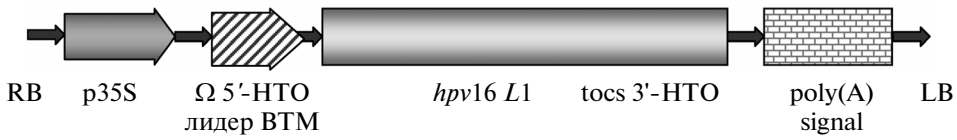

Fig. 1. pBINHPV16 L1 (TMV 5'-UTR Ω) genetic construct used to transform tomato fruit.

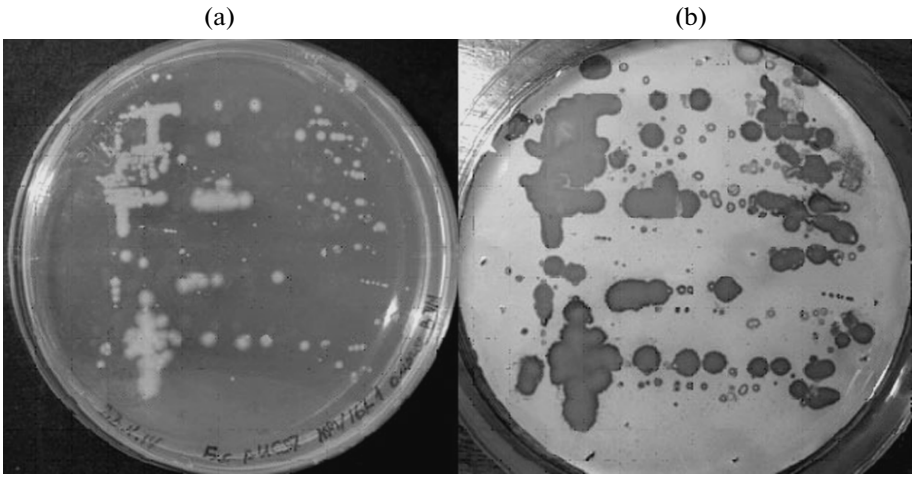

Fig. 2. Results of Western blot analysis of *E. coli* XL1-Blue colonies with the pUC57 plasmid carrying the HPV16 L1 TMV 5'-UTR Ω genetic construct. The general view of the colonies grown on LB medium supplemented with 50 mg/L kanamycin (a). Western blot of colonies (b). Murine antibodies to HPV16 L1 (Genway Biotechnology, United States) were used as primary antibodies.

inserted in the 5'-untranscribed region of the construct. The octopine synthase *tocs* gene from *Agrobacterium* T-DNA was used as a terminator. The construct also contained a poly(A) signal in the 3'-untranscribed region. This construct was inserted into the pBINPLUS/ARS binary plasmid between the RB and LB flanking sequences of T-DNA, which carried the *nptII* gene, coding for neomycin phosphotransferase, as a selectable gene for selection of *Agrobacterium* colonies with the target construct.

For storage and cloning, the pUC57HPV16 L1 (TMV 5'-UTR Ω) plasmid was first transfected into competent *E. coli* XL1-Blue cells. The colonies were obtained on LB medium supplemented with 50 mg/L kanamycin (Fig. 2a) by plating in Petri dishes 150 mm in diameter. To detect the expression of the gene coding for HPV16 L1, the colonies appeared after 16–18 h of growth were covered with sterile nitrocellulose membrane filters (Amersham, United Kingdom) impregnated with 10 mM isopropyl-β-thiogalactopyranoside, a non-substrate inducer of the β–galactosidase gene. After incubation on a bacterial lawn for 6 h, the membrane filters were removed (the positions of colonies were preliminarily marked by pricking with a sterile needle), rinsed in a buffer containing 10 mM Tris-HCl (pH 8.0), 150 mM NaCl, and 0.05% Tween-20, and placed for 1 day in a blocking solution containing 3% bovine serum albumin with 0.01% sodium azide to prevent bacterial contamination.

As can be seen in Fig. 2, all colonies germinated on the selective medium (Fig. 2a) within 16–18 h expressed the target protein HPV16 L1 (Fig. 2b).

To obtain transgenic plants, *A. tumefaciens* EHA105 competent cells were transfected with the pBINHPV16 L1 (TMV 5'-UTR Ω) plasmid and plated on YEP medium supplemented with 50 mg/L kanamycin. After 30 h of culturing, the colonies were obtained in an amount of 25–40 colonies per plate. The presence of the protein antigen in the *A. tumefaciens* EHA105 colonies with pBINHPV16 L1 was selectively estimated by Western dot blot hybridization (Fig. 3).

Genetic transformation was performed by injecting the suspension of *A. tumefaciens* EHA105 with pBINHPV16 L1 (TMV 5'-UTR Ω) immediately to tomato fruit.

As can be seen in Fig. 4, the content of HPV16 L1 protein antigen as a result of this transformation was sufficiently high and amounted to 287 ng/mg total soluble protein (TSP). The maximum result (2330 ng/mg TSP) was obtained with the fruit of the F1 hybrid "Rozovyi flamingo." It should be noted that, in the previous studies without using the omega leader sequence, the content of the protein antigen in tomato fruit was only 50–70 ng/mg TSP in the case of the TBI-HBS antigen and 130–211 ng/mg TSP in the case of the PreS2-S antigen [5, 6].

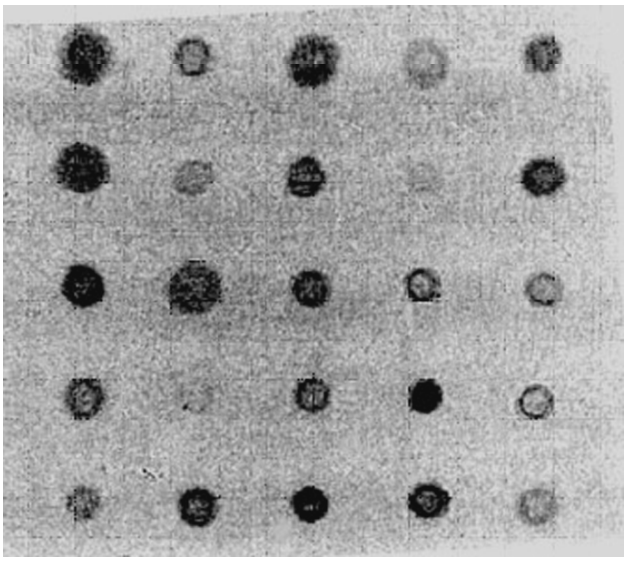

Fig. 3. Western dot blot analysis of the HPV16 L1 protein antigen from the *A. tumefaciens* EHA105 colonies with pBINHPV16 L1 TMV 5'-UTR Ω, which were obtained by selection on YEP medium supplemented with 50 mg/L kanamycin. Blots were developed using the ECF Western Blotting Kit (Amersham, United Kingdom). The primary antibodies to HPV16 L1 were from GenBio Tech (United States), and the secondary antibodies (alkaline phosphatase conjugates) were from Sigma (United States).

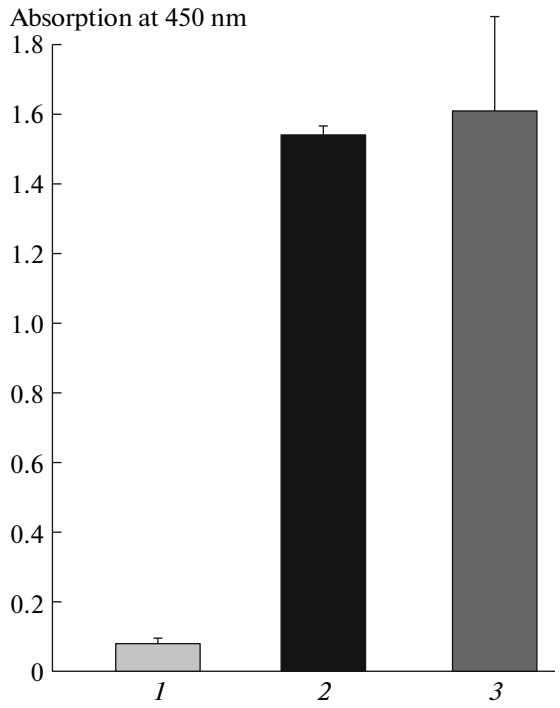

Fig. 4. ELISA analysis of the content of the HPV16 L1 protein antigen: (*1*) untransformed control (n = 6); (*2*) HPV16 L1 standard 200 ng (GenBio Tech), $n = 10$; and (*3*) genetic transformation of tomato fruit, $n = 40$. Data are represented as $M \pm m$.

Thus, the use of the TMV 5'-UTR Ω sequence as a translation amplifier significantly increased the yield of the HPV16 L1 protein antigen as compared to the previously used constructs without the TMV 5'-UTR Ω sequence, which provides the possibility of obtaining a vaccine material for preclinical trials of the candidate vaccine obtained.

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