NUCLEOTIDE SEQUENCE AND RECOMBINANT EXPRESSION IN Escherichia coli OF COAT PROTEIN GENE FROM A CUBAN ISOLATE OF TYLCV

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Introduction

Typical symptoms of TYLCV (tomato yellow leaf curl virus) infections in tomato plants have been observed in Cuba since 1987 (1). TYLCV, a whitefly transmitted geminivirus, became the most devastating disease of tomato in Cuba, causing up to 100 % crop loss. Here, we report the nucleotide sequence of coat protein (*cp*) gene and its expression in *E. coli* in order to characterize the TYLCV Cuban isolate (TYLCV-Cu) and obtain antiserum against it.

Materials and Methods

The DNA was isolated from 2 g of infected plant leaf tissue using Dellaporta method (2). The *cp* gene was amplified by PCR and cloned in pBlueScript (Sk+) (Stratagene, Germany) and pQE30 (QUIAGEN, Germany) plasmids for its sequencing and its expression, respectively. The nucleotide sequence was compared with *cp* gene from a large number of geminiviruses either New and Old World. The phylogenetic tree was obtained using TreeAlign (3). For the expression, the recombinant plasmid pQE30 TYLCVCP was transformed in *E. coli* strain SG 13009. After the induction with 2 mM of IPTG, the proteins were separated by 10 % SDS PAGE. The expression was confirmed by Western blotting with antiTYLCV Sardinia isolate (TYLCV-S) antibodies. The recombinant protein, produced as inclusion bodies, was purified using Ni-NTA resin after the extraction with phosphate buffer, pH 8 containing 6 M guanidium.

Results

A DNA fragment of 777pb (*cp* gene) was obtained by PCR. The *cp* nucleotide sequence comparison with other geminiviruses showed the highest homology (96.2 %) with the TYLCV from Israel (TYLCV-I)(4) suggesting that TYLCV from Cuba should be considered a strain of TYLCV-I (5). Figure 1 shows the phylogenetic relationship with other geminiviruses. The expression level of recombinant protein (33 kD instead of 28 kD expected due to 6 His tag) reached 20 % of total cellular proteins (Figure. 2). Metal affinity chromatography using Ni-NTA resin was efficient for the purification of expressed protein. The purified TYLCV CP will be used to immunize rabbits.

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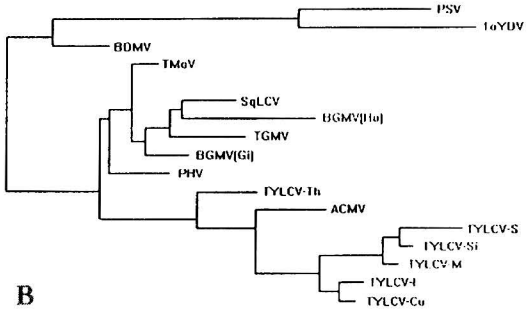

Figure 1. *Phylogenetic tree obtained from alignment of 16 geminiviruses* cp *gene.*

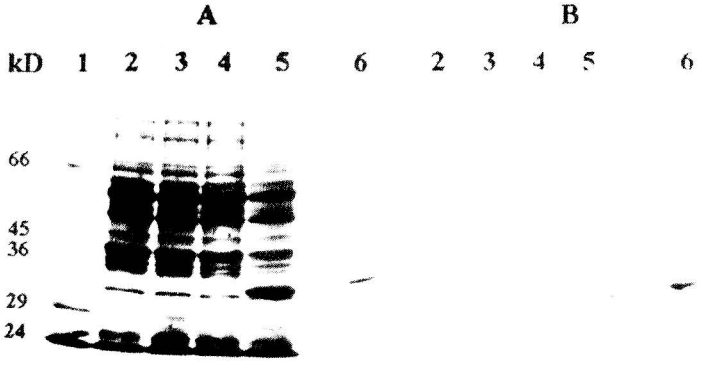

Figure 2. *Expression of TYLCV CP in* E. coli. *A- 10 % SDS PAGE. B- Western blotting using antibodies against TYLCV Sardinia isolate.*
1-Molecular weight marker. 2- pQE30 no induced. 3- pQE30 induced. 4- pQE30 TYLCV CP no induced. 5- pQE30TYLCV CP induced. 6- Purified recombinant TYLCV CP