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 antisera against it.

Materials and Methods
The DNA was isolated from 2 g of infected plant leaf tissue using Dlallaporto method (2). The cp gene was amplified by PCR and cloned in pBlueScript (Sk+) (Stratagene, Germany) and pQE30 (QULAGEN, 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 using anti-TYLCV 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 5 M guanidium.

Results
A DNA fragment of 777 pb (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 kDa instead of 28 kDa 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.

Figure 1. Phyllogenetic 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-pQE30 TYLCV CP induced. 6-Purified recombinant TYLCV CP