Cloning, sequencing and expression of the coat protein gene of Cocksfoot mottle virus

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Abstract  The coat protein (CP) gene of Cocksfoot mottle virus (CFMV) was amplified by RT-PCR and inserted into expression vector pGEX-4T-1, and the resulting plasmid was designated as pGEXCMV-JANCP. The fusion protein GST-CP was expressed in BL21 (DE3) pLysS after IPTG induction. The results of SDS-PAGE and Western blot analysis showed that the CFMV-CP gene was efficiently expressed in E. coli BL21 (DE3) pLysS through IPTG induction and the 56.6 kD protein was obtained.

Keywords: Cocksfoot mottle virus, sequence analysis, prokaryotic expression.

Cocksfoot (Dactylis glomerata L.) is a cool-season gramineous grass that has been planted in North Africa for about 200 years[1]. Recently, it has been widely grown in some provinces of China such as Sichuan, Shanxi, Gansu, Heilongjiang and has exhibited remarkable economic and environmental importance. CFMV is one of the major viruses that caused cocksfoot mosaic, mottle, yellowing and necrotic diseases[2-6] and it is a member of the genus Sobemovirus[7].

CFMV was first described by Makinen et al.[8], then was isolated by Ryabov[6] et al. and Toriyama[9] et al. from Russia and Japan respectively. So far, this virus has not been found in China. Therefore we cloned, sequenced, and expressed CFMV CP gene using a Japanese isolate.

1 Materials and methods

1.1 Virus preparation

The virus CFMV-JAN was isolated from a Dactylis glomerata L. plant grown in Japan, then transferred by mechanical inoculation to barley (Chunxing) or wheat (Nonglin 61) seedlings. CFMV infected leaves were ground in 0.3 mol/L phosphate buffer (pH 6.0) containing 3 mmol/L EDTA on ice, then homogenized and filtered through cheesecloth, and the supernatant was treated with 20% 1-butanol and chloroform (1:1). The virus was collected by centrifugation, and was further purified by ultracentrifugation on 10%—40% linear sucrose density gradient.

1.2 Cloning of CP gene

The RNA was extracted from purified virus by phenol: chloroform (24:1) extraction and dissolved in 30 μL of DEPC H2O. The first strand cDNA from viral RNA was synthesized with specific primers using the first strand cDNA synthesis kit (Promega). A pair of primers for PCR was designed according to the CFMV CP gene sequence deposited in GenBank (AB040447): forward primer 5'-GGGGATCCATGGTGAGGAAAGGAGCA-3', which contains a BamH I site (underlined) and reversed primer 5'-GC GAGCTCCTGCGGTACTAATAC-3', which contains a Sac I site (underlined). PCR conditions were 30 cycles of 94℃ 5 min, 94℃ 1 min, 52℃ 1 min, 72℃ 1 min, and an additional extension at 72℃ for 10 min. The products of PCR were purified with Tiangen PCR preps DNA purification kit, then digested with BamH I and Sac I and cloned into a similarly digested cloning vector pUC19 and its nucleotide sequence was confirmed by double digestion.
with *Bam*H I and Sac I and sequencing analysis.

1.3 Sequence analysis of the *CP* gene

The homology searches were performed with BLAST at http://www.ncbi.nlm.nih.gov/blast/ and the CP sequences were analyzed by BioEdit software.

1.4 Construction of vector and expression of recombinant protein in *E. coli*

A DNA fragment encoding the whole coding region of CP gene was digested with *Bam*H I and EcoR I. The digested DNA fragment was inserted into the *E. coli* expression vector PGEX-4T-1 (Pharmacia) and its nucleotide sequence was confirmed by sequencing. The recombinant vector was transformed into *E. coli* BL21 (DE3) pLys competent cells. Aliquots of overnight cultured transformants were grown in LB medium at 37 °C in the presence or absence of 1.0 mmol/L isopropyl-β-D-galactoside (IPTG). The optical density at 600 nm (OD₆₀₀) was measured every 30 min during the incubation. Expression of the recombinant protein was induced by adding IPTG to the media at a concentration up to 1.0 mmol/L at 37 °C when OD₆₀₀ of the culture reached 0.5. *E. coli* cells were harvested every 2 h of incubation and disrupted by sonication and the soluble fraction of the lysate was collected. The recombinant protein was separated by 12% SDS-PAGE and the protein was either stained with Coomassie brilliant blue R-250 or transferred for Western blot analysis by a routine method.

2 Results and discussion

2.1 Detection of the recombinants

Fig. 1(a) shows a 792 bp DNA fragment amplified by RT-PCR as we expected. Restriction enzyme digestion of this product generated two DNA fragments (Fig. 1(b)), demonstrating that the whole coding region of *CP* gene was successfully cloned by our cloning strategy.

![DNA marker](attachment:image)

2.2 Sequence analysis of *CP* gene

The sequence analysis revealed that *CfMV-JAN* CP gene (GenBank accession No. AB040447) shared similarities of 97.1% and 95.5%, respectively, to those of *CfMV-FIN* (Z 48630) and *CfMV-RUS* (L40905), and revealed 20 point mutations in the open reading frame of these genes (Fig. 2). Interestingly, most of the mutations occurred at the third nucleotide of codons. The mutations in *CfMV-RUS* CP were found at the positions of 18, 57 and 174 nucleotides, which changed amino acids from Lys to Arg, Arg to Thr and Val to Met respectively. The mutations in *CfMV-JAN* CP were at the positions of 9 and 174, resulting in amino acid changes of from Thr to Ala and Val to Met (Fig. 3).

| *CfMV-FIN* CP | ATGATGGTGAGGAAGAGCGACGAGAAGCCCGGCAACACAAAAACCCAGGCTCAG 60 |
| **CfMV-RUS** CP | 1 | 61 | CAGCAGCGCTGGGGGCGCGCGAGGCCTGCTCGTATGGCAGACCGCTCCCTGACCTGTCGC 120 |
| **CfMV-JAN** CP | 1 | 58 | 58 | 117 |
| **CfMV-FIN** CP | 121 | TTGACCCTCCAGCGCTGACCTTGGTTCGCACTTGAAGGCTGTTAGAGGCAAGACGCTGCTG 180 |
| **CfMV-RUS** CP | 118 | 118 | 117 |
| **CfMV-JAN** CP | 118 | 118 | 117 |
| **CfMV-FIN** CP | 181 | GTGAGTCACTGTTTGGATACCCGCGCATGACACCCAGTTACCTATTGTTGCTTACGGCACA 240 |
| **CfMV-RUS** CP | 178 | 178 | 177 |
| **CfMV-JAN** CP | 178 | 178 | 177 |

*(To be continued on the next page)*
Fig. 2. Sequence alignment of the CfMV-JANC, CfMV-FINCP and CfMV-RUSCP. * Asterisks indicate identical bases.

Fig. 3. Sequence alignment of the deduced amino acid sequences of CfMV-JANC, CfMV-FINCP and CfMV-RUSCP. * Asterisks indicate identical residues.
2.3 Results of SDS-PAGE and Western blot analysis

The positive clones were confirmed by restriction enzyme digestion and sequencing analysis, then transformed into *E. coli* BL21 (DE3) pLysS and protein expression was induced by IPTG. The expressed product, with a molecular weight of 56.0 kD, was detected by both SDS-PAGE (Fig. 4) and Western blot analysis. This result revealed that the fusion protein was the specifically expressed product.

In conclusion, we cloned the CP gene of CMV-JAN and had it efficiently expressed in *E. coli*, which will help us to develop the antibodies against the CMV-CP by the expressed proteins and will accelerate the development of the vaccines against the virus.

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References