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Immunological Detection of Garlic Latent Virus

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Abstract : To understand the molecular structure and pathogenesis mechanism of Korean garlic viruses, we have isolated cDNA clones for garlic viruses. The partial nucleotide sequences of 24 cDNA clones were determined and those of five clones containing poly(A) tail were compared with sequences of other plant viruses. One of these clones, V9, has a primary structure similar to the carlavirus group, suggesting that the clone V9 derived from a part of garlic latent virus (GLV). Northern blot analysis with the clone V9 as a probe demonstrated that GLV genome is 8.5 knt long and has a poly(A) tail. The clone V9 encodes coat protein (CP) of 33 kDa and nucleic acid binding protein of 10 kDa in different reading frame. The hexanucleotide motif, 5'-ACCUAA, which is conserved in the 3' noncoding region and was proposed to be a *cis*-acting element involved in the production of negative strand genomic RNA was noticed. Complementary sequence to the hexanucleotide motif, 5'-TTAGGT, is also found in the positive strand of V9 RNA. The putative CP gene was cloned into the pRSET-A expression vector and expressed in *E. coli* BL21. The expressed recombinant V9CP protein was purified by Ni^{2+}·NTA affinity chromatography. The anti-V9CP antibody recognizes 34 kDa polypeptide which could be CP of GLV in infected garlic leaf extract. Immunoblot and Northern blot analysis of various cultivars shows wide occurrence of GLV in Korean garlic plants(Received January 5, 1995; accepted February 7, 1995).

Introduction

Garlic (*Allium sativum* L.) is an important vegetable crop and has long been cultivated extensively in Korea. Based on the microscopic observation and the symptoms produced in infected garlic plants, two sap-transmissible filamentous viruses were previously described : garlic latent virus (GLV) and garlic mosaic virus (GMV).[1)]

Garlic plants inoculated with GLV produce no visible symptoms in early phase of infection, whereas infected broad beans produce systemic necrotic spots. *Chenopodium amaranticolor, C. quinoa,* and *Tetragonia expansa* infected with GLV produce local necrotic and chlorotic lesions. Particles of GLV are flexuous rods, 650~700 nm long, and exist either randomly or as small aggregates in the cytoplasm of the infected plant cells as is most of the carlaviruses.[1)]

Carlavirus is slightly flexuous filaments, often appearing curved to one side, normally 610~700 nm long and 12~15 nm in diameter. It sediments at 147~176 S. The particles are composed of approximately 1,600~2,000 subunits of a single protein species whose Mr are usually 31,000~34,000. Coat proteins are arranged as a helix (pitch 3.3~3.45 nm) enclosing the genome which is a single molecule of single-stranded RNA (Mr 2.3~3.0 $\times 10^6$). Genomic RNA constitutes normally 5~7% of the particle weight. Infections in natural hosts are often latent, but sometimes mosaic symptoms are produced.[2)]

Present data indicate that most, if not all, of unselected commercial garlic cultivars contain a complex of two or more viruses.[3-5)] The identification of the individual viruses contained in garlic plant is however variable, particularly in materials from diverse geographical locations. Due to the absence of molecular characterization, electronmicroscopic examination and immunological cross-reactivity were the methods of choice available. In this paper we have isolated a cDNA clone for the coat protein (CP) of GLV. It was overexpressed in *E. coli* and a polyclonal antibody was raised against the recombinant CP. The nascent CP of GLV from garlic leaf extract or garlic virus preparation was identified with the antibody and the occurrence of GLV in field-grown garlic is examined.

Materials and Methods

Virus preparation

Virus particles were isolated from garlic leaves showing yellow streak of virus symptom by the procedure described previously.[6)]

Construction of GV cDNA library

Key words : garlic, garlic virus, coat protein, carlavirus, garlic latent virus (GLV), immunoblot
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cDNA library was constructed into pUC18 plasmid vector by the method of Gubler and Hoffman[7] with minor modifications as described in Choi *et al.*[6]

Nucleotide sequencing and recombinant DNA techniques

Nucleotide sequencing was carried out in plasmid pUC 18[8] by the dideoxynucleotide chain-termination method of Sanger *et al.*[9] Universal M13 primers for reverse and forward reactions were used and the reaction products were analyzed by 6 M urea-polyacrylamide gel electrophoresis. Northern blot analysis was performed by the procedure of Kroczek and Siebert.[10] Purification and manipulation of DNA were carried out according to the standard protocol of Sambrook *et al.*[11]

Overexpression and purification of recombinant CP

Overexpression and purification of recombinant CP was carried out according to the protocol provided by Qiagen. The *E. coli* transformants were grown at 37℃ in LB medium containing 50 μg/m*l* of ampicillin. At OD_{600} 0.7~0.9 IPTG was added to a final concentration of 1 mM and cells were incubated for additional 4 hrs. Cells were collected by centrifugation at 4,000 g for 5 min and resuspended in 5 m*l*/g of lysis buffer A (6 M guanidine hydrochloride, 0.1 M NaH_2PO_4, 0.01 M Tris, 0.02 M 2-mercaptoethanol, pH 8.0). The lysate was sonicated and collected by centrifugation at 10,000 rpm for 15 min at 4℃. The supernatant was applied on 0.6 m*l* of nickel-NTA-agarose affinity resin (Qiagen protocol), previously equilibrated in buffer A, washed with 10 column volumes of buffer A, 5 column volumes of buffer B (8 M urea, 0.1 M NaH_2PO_4, 0.01 M Tris, 0.02 M 2-mercaptoethanol, pH 8.0) and 6 column volumes of buffer C (same composition as buffer B except pH 6.3). The recombinant protein was eluted with 10 column volumes of buffer D (same composition as buffer B except pH 5.9), followed by 5 column volumes of buffer E (same composition as buffer B except pH 4.5). The proteins in each fraction were analyzed by 12.5% SDS-PAGE.

Preparation of antibody

Preparation of antibody was carried out by the procedure of Sambrook *et al.*[11] Purified recombinant CP was mixed with equal volume of Freund's adjuvant and injected into rabbit (NewZealand White) 3 times at the interval of 2 weeks. Serum was processed by standard method after bleeding.[12]

Immunoblot analysis

Protein samples were analyzed by SDS-polyacrylamide gel electrophoresis.[6] Blotting of proteins from SDS-PAGE gels onto nitrocellulose paper was carried out by electrotransfer at 0.15 A for 4hrs in 50 mM Tris-glycine, pH 9.1, containing 20% methanol. The nitrocellulose blot was treated essentially according to Burnette[13] except that 5% nonfat dry milk was used instead of BSA as a blocking reagent. The nitrocellulose blot was incubated first with GLV antibody (1 : 250 dilution) and then with goat anti-rabbit second antibody labelled with peroxidase. ECL detection reagents 1 and 2 (Amersham Co.) were used as substrate for peroxidase.

Results and Discussion

Multiple species of cDNA clones for garlic virus were isolated

To isolate a cDNA clone for GLV, a cDNA library for garlic virus was constructed.[6] Oligo(dT) was employed as a primer because carlavirus and potyvirus of 600~800 nm long usually was known to have poly(A)$^+$ tail at the 3' end of their genomes.[14] Different 24 cDNA clones were chosen and their nucleotide sequences were determined. Five out of 24 different clones contained poly(A) tracts of 15~57 nucleotides at their 3' end suggesting that they are cDNA clones from the 3' end of the virus. The insert size of these clones was 2.0 kb (G7), 1.4 kb (V9), 1.5 kb (S83), 1.0 kb (S81) and 0.55 kb (S64). Northern blot analysis revealed that 5 clones strongly bind to the garlic virus RNA isolated from field-grown garlic leaves and to the poly(A)$^+$ RNA purified from total garlic virus RNA by oligo(dT)-cellulose chromatography (data not shown). Therefore, we confirmed that five clones are cDNAs for garlic virus containing poly(A)$^+$ tail. The complete nucleotide sequences of G7, V9, S83, S81 and S64 were determined. These clones have no sequence similarity each other. These results further confirm that there are at least five different kinds of viruses in garlic as expected from previous SDS-PAGE analysis.[6,15] Alternatively GLV may contain at least five different pieces of genomic RNA or subgenomic RNA, which is not the case obviously.

The clone V9 encodes the CP of GLV, a carlavirus

The complete nucleotide sequence of the clone V9 was determined (Fig. 1). Its primary structure is similar to the carlavirus group such as lily symptomless virus (LSV) and potato virus S (PVS). The clone V9 consists of two open reading frame(ORF)s. The first ORF located at nucleotides 55~945 encodes CP of Mr 33,000. In case of carlavirus and potyvirus, the average Mr of CP is known to be 31,000~34,000.[2,16] The second ORF located at nucleotides 942~1232 encodes a protein of Mr 10,000. The deduced 10 kDa protein, like the analogous proteins of other carlaviruses, contains a highly conserved region which contains a basic arginine-rich domain and a puta-

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1    agcaagcaaaacctttggttcactTTAGGTatacagcgctctaaattgatatttATGGCTAACGAAGAA   69
1                                                    M  A  N  E  E             5

70   GAAGAACTCAATAACGTTCAGAATTTGCCGACTCGCGACCCTGGGACTATCCCAGAGCATGAGCAGACG   138
6    E  E  L  N  N  V  Q  N  L  P  T  R  D  P  G  T  I  P  E  H  E  Q  T     28

139  AAAGCAGTGAATGATGTCGGCGTTATGGAGCGTGAGGGTTTCGAAGCCGTGCTACGAAGGACGGAAAAC   207
29   K  A  V  N  D  V  G  V  M  E  R  E  G  F  E  A  V  L  R  R  T  E  N     51

208  AGATTCAACAAACTTAAGGAAAAGTGCATGTCCGAATTATCCAGCGTGAATGTCACGAATTGCGGGTGG   276
52   R  F  N  K  L  K  E  K  C  M  S  E  L  S  S  V  N  V  T  N  C  G  W     74

277  GAGTCTGGACGCCCAAAAGCACAACTAGCTGACAGTCTCAAAGGTGACGCTTCTAATATTTTCACCCGC   345
75   E  S  G  R  P  K  A  Q  L  A  D  S  L  K  G  D  A  S  N  I  F  T  R     97

346  CCTTCTATGGATGCCCTCCTTGTTCGGAATTACGCACCTGAAAGTAACAACATGGCCACTGCTGAGGAA   414
98   P  S  M  D  A  L  L  V  R  N  Y  A  P  E  S  N  N  M  A  T  A  E  E    120

415  TTGGCAAAAATCTCCGCTAAGGTGCAGGCTCTTGGTGCCCCTGAAGAATGTTTAGCTGAAGTGTTCTGG   483
121  L  A  K  I  S  A  K  V  Q  A  L  G  A  P  E  E  C  L  A  E  V  F  W    143

484  GATATATGCATGTATTGCACCACTGCTGGAAGTTCTCCAAATGTAAATCCTAAAGGAACTATCTCTGTT   552
144  D  I  C  M  Y  C  T  T  A  G  S  S  P  N  V  N  P  K  G  T  I  S  V    166

553  GGTGGCAAGGTCGTTACTAGAGACATGGTTGTAGCTGTCATCAAAGAGTACTCCACATTACGCCAGGTC   621
167  G  G  K  V  V  T  R  D  M  V  V  A  V  I  K  E  Y  S  T  L  R  Q  V    189

622  TGCCGCTGCTACGCGCCTGTGGTCTGGAACTACATGTTATTGAATGAGCAACCACGCCAATTGGGAC   690
190  C  R  C  Y  A  P  V  V  W  N  Y  M  L  L  N  E  Q  P  P  A  N  W  D    212

691  GCAAAAGGTTTTACTGAGAACACTAAATATGCTGCCTTTGACACTTTGATGCTGTCACGAATAAAGCT   759
213  A  K  G  F  T  E  N  T  K  Y  A  A  F  D  T  F  D  A  V  T  N  K  A    235

760  GCAATTCAACCTCTTGAGGGCCTGATTAGGGCCCCCACTGATGCAGAACGAATTGCCTTTGCAACGCAT   828
236  A  I  Q  P  L  E  G  L  I  R  A  P  T  D  A  E  R  I  A  F  A  T  H    258

829  AAAAAGTTGGCTCTAACCAAAAATTCTCAGAATGCTCGTTACGCCAATACTTCTGCTGATGTTACTGGA   897
259  K  K  L  A  L  T  K  N  S  Q  N  A  R  Y  A  N  T  S  A  D  V  T  G    281

898  GGTTTCTTCGGCTGTTTTCCAAAAAATAATTTCAGAGAGAATCGATGTTGATCAAACAGAGAACTTACC   966
282  G  F  F  G  C  F  P  K  N  N  F  R  E  N  R  C  *                      298
1                                                    M  L  I  K  Q  R  T  Y     8

967  GTAGGCTACTGCGTGTTATATTTAAATTACATACTAATAAGAATTGTGTGGATCTGATAAATATAATCG   1035
9    R  R  L  L  R  V  I  F  K  L  H  T  N  K  N  C  V  V  L  I  N  I  I     31

1036 TGAGTAAAATAGTGTGTGAAAGTGCGGGTGCTTCGAATGCTCGAGCTCGTAGGGCTAAGAGCATAGGCA   1104
32   V  S  K  I  V  C  E  S  A  G  A  S  N  A  R  A  R  R  A  K  S  I  G     54

1105 GGTGCCCTCGCTGTTTTCGGTGTTCACCTGGTTTTTATTTCACTAAAAACTGTGACACGAAAAATTGTT   1173
55   R  C  P  R  C  F  R  C  S  P  G  F  Y  F  T  K  N  C  D  T  K  N  C     77

1174 CCGCAGGTATTAACTATAATGCAAAAGTCAAGGATTTTATAGTTGATGGTGTAACAATGTGAaacccta   1242
78   S  A  G  I  N  Y  N  A  K  V  K  D  F  I  V  D  G  V  t  M  *           98

1243 ctacaaaccttggctagttgcccataaaACCTAAgtaatgcataagtgggaacgtataaaatgaatttgt   1311
1312 ttttaaaatattttcgcaaaaaaaaaaaaaaaaa                                      1346
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Fig. 1. Nucleotide sequence and deduced amino acid sequence of the clone for garlic latent virus. CP gene corresponds to the first open reading frame from the nucleotide 55-945. The second open reading frame encodes the 10 kDa protein. The hexanucleotide sequence, ACCTAA, a *cis*-acting element for viral RNA replication, is underlined and TTAGGT complementary to ACCTAA is in reverse.

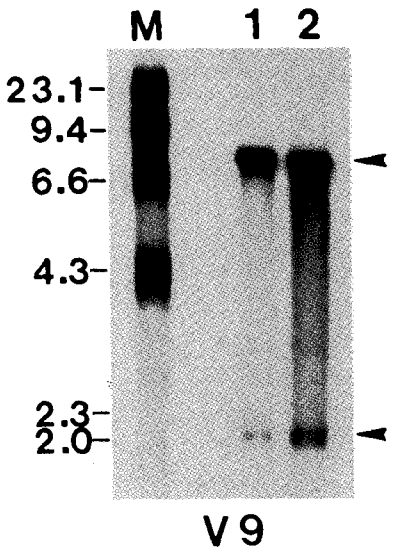

Fig. 2. Northern blot analysis of garlic virus RNA. RNA was separated by 0.8% formaldehyde agarose gel electrophoresis and transferred onto nylon membrane. The filter was probed with random primer-extended clone V9. Lane M, λ/HindIII size marker; lane 1, garlic virus RNA from field-grown garlic leaves; lane 2, poly(A)$^+$ RNA purified from garlic virus RNA by oligo(dT)-cellulose chromatography

tive zinc finger motif.[17] Analogous 'finger' structures are found in other plant virus-specific proteins[18] and interestingly in case of potato virus M and chrysanthemum virus B, they bind to nucleic acids *in vitro*.[19]

In carlavirus a hexanucleotide motif (5'-ACUUAA) is conserved in the 3' noncoding region. This sequence was proposed to be involved in the production of negative strand genomic RNA by functioning as a *cis*-acting element involved in replicase recognition of the template.[20] The similar sequence (5'-ACCUAA) is found in the 3' noncoding region of the clone V9. This variant sequence motif was also noticed in other carlaviruses, Helenium virus S (HelVS) and LSV.[20] The same hexanucleotide motif (5'-ACCUAA), containing a highly conserved C at position 3, is also present in the negative strand of carlavirus RNAs in regions where 5' termini of subgenomic RNAs for the 25 kDa product (ORF2) and CP (ORF5) of carlaviruses are predicted to map.[20] Complementary sequence, 5'-TTAGGT, was also found in the positive strand of V9 RNA. The presence of the hexanucleotide motif in regions where viral RNA synthesis is believed to initiate provides additional support that the motif plays a functional role in viral RNA replication.[20] To look for the RNA transcript corresponding to the isolated cDNA clone, V9, Northern blot analysis was carried out with the clone V9 as a molecular probe (Fig. 2). Garlic virus was isolated from field-grown garlic leaves showing yellow streak. Garlic virus RNA containing poly(A)$^+$ tail was purified by oligo(dT)-cellulose chromatography. From garlic virus RNA about 44% of poly(A)$^+$ garlic virus RNA was recovered in oligo(dT)-cellulose chromatography. Virus RNA and poly(A)$^+$ RNA from garlic virus RNA preparation show strong signal at the position of 8.5 knt and 1.5 knt. Another band of 2.6 knt was also detected but with variable intensity. The strong signal at the position of 8.5 knt corresponds to GLV genome, but about 1.5 knt long transcript seems to be a subgenomic RNA encoding CP. It is known that GLV particles are flexuous rods, 650~700 nm long as is most of the carlavirus.[1] The genome size of carlaviruses are approximately 7.5 knt.[21] The size of the detected genomic and subgenomic RNA were 7.5 knt, 2.5 knt and 1.3 knt in the case of PVS[22], and 7.4 knt, 3.3 knt and 1.5 knt in the case of HelVS.[23] In addition to the major population of virus particle of approximately 660 nm in length, two smaller secondary peaks were often evident in PVS and HelVS.[22-23] The presence of smaller particles suggest that subgenomic RNA may be encapsidated in these smaller particles.[21] Our results also demonstrated that the virus genome corresponding to the clone V9 has a poly (A) tail like in most of other single-

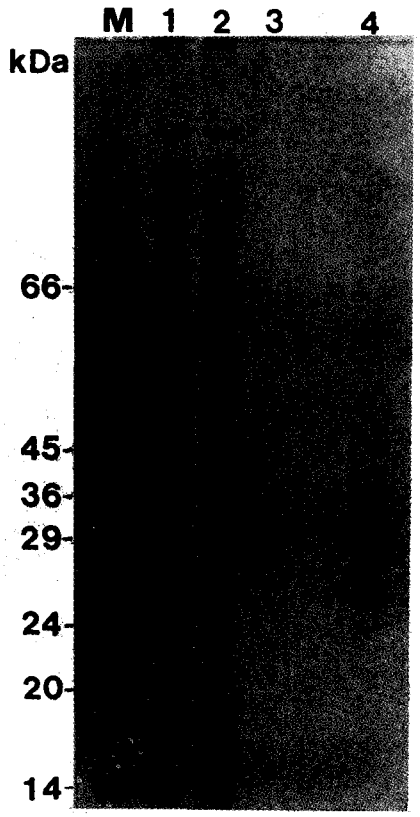

Fig. 3. SDS-PAGE analysis of GLV CP expressed in *E. coli* BL21. The expressed CP was purified by Ni^{2+}·NTA affinity chromatography. Lane 1, Cell lyzate of *E. coli* with vector only; lane 2, IPTG-induced *E. coli* transformed with the pRAT12; lane 3, Ni^{2+}·NTA-purified recombinant CP; lane 4, garlic virus preparation from garlic leaves showing yellow streak

stranded RNA virus in plants.[14]

Taken together, we conclude that the clone V9 encodes the CP of GLV, a carlavirus. These results are consistent with Nagakubo *et al.*[24] A partial cDNA clone, GV1, shows 93% homology in amino acid sequence with the clone V9. But there is a sequence entry in EMBL Data-Base which shows 93% homology with the clone V9. It was named GMV[25] even though the rationale is not clear.

Identification of the CP for GLV

To identify the CP of GLV, cDNA fragment (nucleotides 227~1346) was cloned into the pRSET-A expression vector and expressed in *E. coli*. The pRSET vector allows high level expression of foreign gene in *E. coli* by the T7 promoter. The recombinant construct pRAT12 was transformed into *E. coli* BL21 and T7 RNA polymerase was induced by IPTG. The expressed recombinant CP of GLV was purified by Ni^{2+}·NTA affinity chromatography by the help of an N-terminal metal binding domain on the fusion peptide provided by the pRSET vector. The expressed protein was analyzed by 12.5% SDS-PAGE. The IPTG-induced *E. coli* transformed with the pRAT12 expressed the expected 30 kDa polypeptide of recombinant protein. The recombinant CP was purified to homogeneity (Fig. 3).

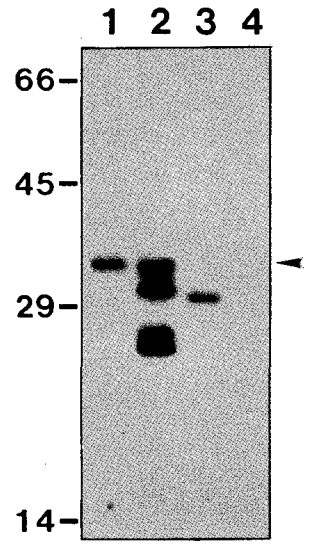

Fig. 4. Immunoblot analysis of CP. Protein samples were separated on 12.5% SDS-PAGE, transferred onto nitrocellulose paper and probed with anti-CP antibody for GLV. Arrowhead indicates the nascent CP of GLV. Lane 1, garlic leaf extract; lane 2, garlic virus preparations from cultivar Danyang; lane 3, affinity-purified recombinant CP; lane 4, affinity-purified 10 kDa protein

Polyclonal antibody was raised against the purified protein

To find out the specificity of anti-V9 antibody, immunoblot analysis was carried out (Fig. 4). The anti-V9 antibody recognizes a band of Mr 34,000 from infected garlic leaf extract (Fig. 4, lane 1) demonstrating the specificity of the antibody. It also recognizes recombinant V9 antigen (Fig. 4, lane 3). With garlic virus preparation from garlic cultivar Danyang three additional bands of Mr 32,000, 26,000 and 24,000 were detected. It is possible that the lower bands are be degradation products of 34 kDa CP. The lengthy purification procedure may allow degradation of the CP by proteolytic enzymes. To test this possibility, garlic leaf extract was incubated at 37℃ in SDS-sample buffer for various length of time and each fraction was analyzed by immunoblotting (data not shown). The decrease of the intensity of 34 kDa band and the increase of those of lower bands of 32, 26 and 24 kDa support that they are proteolytic cleavage products of GLV CP. The proteins of some carlaviruses can become partially degraded in the assembled particles.[2] It is also known that the N- and C-termini of CP of potyvirus are degraded during purification and storage by enzymes of plant origin which cosediment with the virus particles.[26] Storage of a purified preparation of tobacco etch virus at 4℃ resulted in the conversion of CP from Mr 32,000 to 26,000.[27]

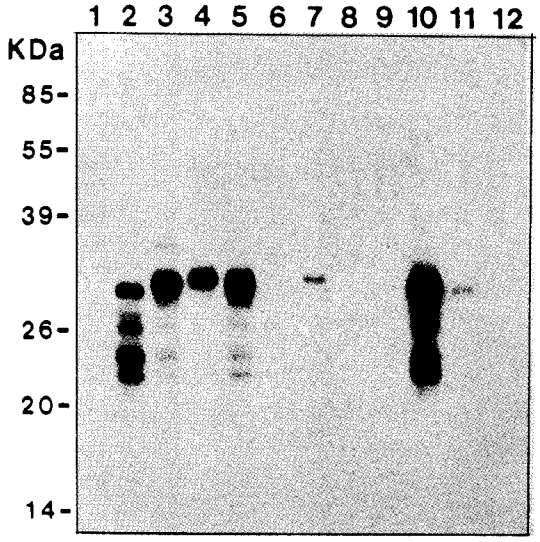

Fig. 5. Immunoblot analysis of CP from various cultivars of garlic. Protein samples were separated on 15% SDS-PAGE, transferred onto nitrocellulose paper and probed with anti-CP antibody for GLV. Each sample is garlic leaf extract of tissue-cultured garlic(lane 1, 1.5 mg), field-grown garlic(lane 2, 0.75 mg), Usung(lane 3, 0.5 mg), Danyang 1(lane 4, 0.5 mg), Danyang 2(lane 5, 0.5 mg), Danyang 3 (lane 6, 1.5 mg), Wonju(lane 7, 1.0 mg), Seosan(lane 8, 1.5 mg), Namhae(lane 9, 1.5 mg), elephant garlic(lane 10, 0.4 mg), Oswego-white(lane 11, 1.0 mg) and Namdo(lane 12, 1.5 mg).

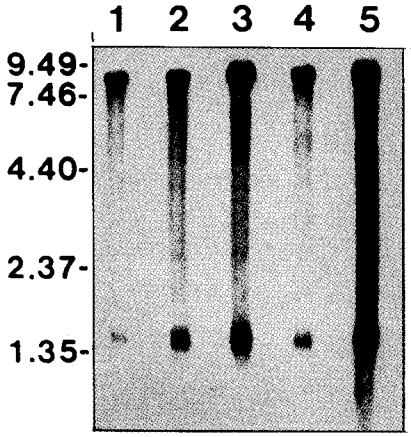

Fig. 6. Northern blot analysis of garlic virus RNA from various cultivars of garlic. RNA was separated by 0.8% formaldehyde agarose gel electrophoresis and transferred onto nylon membrane. The filter was probed with random primer-extended clone V9. Each sample is garlic virus RNA of Eosangcheon (lane 1), Daegang (lane 2), Wonju (lane 3), Euiseong 1 (lane 4) and Euiseong 2 (lane 5).

Occurrence of GLV in Korean garlic plants was examined

To understand the occurrence of GLV in various cultivars of Korean garlic, immunoblot analysis was employed. Garlic leaf extract was prepared from each cultivar of garlic. Protein samples were separated on 15% SDS-PAGE, transferred onto nitrocellulose paper and probed with anti-CP antibody for GLV (Fig. 5).

As shown in Fig. 5 GLV CP was variable in size from 32 to 34 kDa among different cultivars and strains. The lower bands in lane 2 and 10 of Fig. 5 could be proteolytic cleavage products of GLV CP as mentioned above. Molecular weight of carlavirus CP is known to be in the range of 31~34 kDa[2]. The GLV CP is also variable in quantity among different cultivars. Very weak signal, if any, was detected from the tissue-cultured garlic (lane 1) as expected. In case of cultivar Danyang 3, Seosan and Namhae, the signals were also very weak. Considering that the amount of sample analyzed, the elephant garlic has the highest signal to the CP antibody for GLV. From these results, we conclude that GLV is variable both in size and in the degree of infection among cultivars and strains. These results are consistent with Northern blot analysis (Fig. 6). Intensity of the GLV band from various cultivars is rather variable. From the above results, we suggest that GLV is a major virus infecting garlic cultivated in Korea.

Acknowledgement

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References

1. Lee, Y. W., S. Yamazaki, T. Osaki and T. Inouye (1979) Two elongated viruses in garlic, garlic latent virus and garlic mosaic virus. *Ann. Phytopath. Soc. Japan* **45**, 727-734.
2. Koenig, R. (1982) Carlavirus group. *CMI/AAB Descriptions of Plant Viruses* No. 259.
3. Cadilhac, B., J. B. Quiot, J. Marrow and J. P. Leroux (1976) Mise en evidence au microscope electronique de deux virus differents infectant l'ail(*Allium sativum*) et l' echalotte(*Allium cepa var. ascalonicum*). *Ann. Phytopath.* **8**, 65-72.
4. Chang, M. U., W. W. Park, J. D. Chung, K. B. Lim and Y. J. La (1988) Distribution of garlic latent virus and garlic mosaic virus in infected garlic tissues. *J. Kor. Soc. Hort. Sci.* **29**, 253-265.
5. Walkey, B. G. A., M. J. W. Webb, C. J. Bolland, and A. Miller (1987) Production of virus-free garlic (*Allium sativum* L.) and shallot (*A. ascalonicum* L.) by meristem-tip culture. *J. Hortic. Sci* **62**, 211-220.
6. Choi, J. N., J. H. Ahn, Y. D. Choi and J. S. Lee (1993) Molecular cloning of cDNAs for Korean garlic viruses. *J. Kor. Agric. Chem. Soc* **36**, 315-319.
7. Gubler, U. and B. J. Hoffman (1983) A simple and very efficient method for generating cDNA libraries. *Gene* **25**, 263-269.
8. Vieira, J. and J. Messing (1982) The pUC plasmids, an M13 mp7-derived system for insertion mutagenesis and seqencing with synthetic universal primers. *Gene* **19**, 259-268.

9. Sanger, F., S. Nicklen and A. R. Coulson (1977) DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**, 5463-5467.
10. Kroczek, R. A. and E. Siebert (1990) Optimization of Northern analysis by vacuum-blotting, RNA transfer, visualization and ultraviolet fixation. *Anal. Biochem.* **184**, 90-95.
11. Sambrook, J., E. F. Fritsch and T. Maniatis (1989) Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor Laboratory Press.
12. Garvey, J. H., N. E. Cremer and D. H. Sussdorf (1980) Separation and preservation of serum. *Meth. Immun.* **3**, 36-38.
13. Burnette, W. N. (1981) "Western blotting": Electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal. Biochem.* **112**, 195-203.
14. Huisman, W., H. J. M. Linthorst, J. F. Bol and B. J. C. Cornelissen (1988) The complete nucleotide sequence of potato virus X and its homologies at the amino acid level with various plus-stranded RNA viruses. *J. Gen. Virol.* **69**, 1789-1798.
15. Song, J. T., J. N. Choi, S. I. Song, J. S. Lee and Y. D. Choi (1995) Identification of a potexvirus in Korean garlic plants. *J. Kor. Agric. Chem. Soc.* **38**, 55-62.
16. Hollings, M. and A. A. Brunt (1981) Potyvirus group. *CMI/AAB Descriptions of Plant Viruses* No. 245.
17. Klug, A. and D. Rhodes (1987) 'Zinc fingers':a novel protein motif for nucleic acid recognition. *Trends in Biochem. Sci.* **12**, 464-469.
18. Sehnke, P. C., A. M. Mason, S. J. Hood, R. M. Lister and J. E. Johnson (1989) A 'zinc finger'-type binding domain in tobacco streak virus coat protein. *Virology* **168**, 48-56.
19. Gramstat, A., A. Courtpozanis and W. Rohde (1990) The 12 kDa protein of potato virus M displays properties of a nucleic acid-binding regulatory protein. *FEBS Lett* **276**, 34-38.
20. White K. A., J. B. Bancroft and G. A. Mackie (1992) Mutagensis of a hexanucleotide sequence conserved in potexvirus RNAs. *Virology* **189**, 817-820.
21. Foster, G. D. (1992) The structure and expression of the genome of carlaviruses. *Res. Virol.* **143**, 103-112.
22. Foster, G. D. and P. R. Mills (1990) Evidence for the role of subgenomic RNAs in the production of potato virus S coat protein during *in vitro* translation. *J. Gen. Virol.* **71**, 1247-1249.
23. Foster, G. D. and P. R. Mills (1990) Evidence for the role of subgenomic RNA species in the production of *Helenium* virus S coat protein during *in vitro* translation. *Virus Res.* **17**, 61-70.
24. Nagakubo, T., M. Kubo and K. Oeda (1994) Nucleotide sequence of the 3' regions of two major viruses from mosaic-disessed garlic: Molecular evidence of mixed infection by a potyvirus and a carlavirus. *Phytopathology* **84**, 640-645.
25. Sumi, S. (1992) EMBL AC:D11161.
26. Shukla, D. D., P. M. Strike, S. L. Tracy, H. K. Gough and C. W. Ward (1988) The N and C termini of the coat proteins of potyviruses are surfaces located and the N terminus contains the major virus-specific epitopes. *J. Gen. Virol.* **69**, 1497-1508.
27. Hiebert, E., J. H. Tremaine and W. P. Ronald (1984) Theeffect of limited proteolysis on the amino acid composition of five potyviruses and on the serological reaction and peptide maps of the tobacco etch virus capsid protein. *Phytopathology* **74**, 411-416.

마늘 잠복 바이러스의 면역학적 진단

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초록 : 한국 마늘에 감염된 바이러스의 종류와 병 발생 메카니즘을 구명하기 위하여, 마늘 바이러스 cDNA clone들을 분리하였다. 24개 cDNA clone들의 부분적인 염기 서열을 결정하였고, 이 중 poly(A) tail을 가진 5개 clone들의 염기 서열을 결정하였다. 이를 이미 알려진 다른 식물 바이러스와 비교했을 때, clone V9은 일차구조가 carlavirus와 유사성을 보이므로 GLV cDNA clone으로 여겨진다. Northern blot 결과로부터 GLV genome의 크기는 8.5 knt이고, poly(A) tail을 가지고 있다는 것을 알 수 있었다. clone V9의 3' 말단부분에는 바이러스 복제과정에서 *cis*-acting element로 작용한다고 여겨지는 hexanucleotide motif(5'-ACCUAA)가 존재한다. 또한 carlavirus의 껍질 단백질 subgenomic RNA의 5' 말단에 보존되어 있는 5'-TTAGGT도 나타난다. 이들은 모두 carlavirus의 특징들이다. 껍질 단백질 유전자를 pRSET-A 발현 벡터에 재조합하고, *E. coli* BL21에서 발현시켰다. 발현된 껍질 단백질을 Ni^{2+}·NTA affinity chromatography에 의해 정제하였다. 껍질 단백질을 토끼에 주사하여 항체를 만든 후, immunoblot을 한 결과 GLV 껍질 단백질에 해당하는 34 kDa polypeptide가 인지되었다. 또한 다양한 마늘 품종에 대해서 immunoblot을 실시한 결과, GLV 껍질 단백질의 크기와 GLV의 감염정도가 마늘 품종에 따라서 차이가 있다는 것을 알 수 있었다.

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