Production and characterization of monoclonal antibodies against tomato ringspot virus

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Tomato ringspot virus (TmRSV) is a kind of dangerous virus which has't been recorded in China. Its host range is wide. We obtained the virus from abroad and produced the monoclonal antibodies (McAbs) against TmRSV by the technique of hybrodoma.

Six monoclonal antibodies to TmRSV were obtained by fusion of SP2/0 myoloma cell with spleen cell from BALB/c mice immunized with TmRSV. They are CG8, 7C10, F9B9, 9E11, 9G9 and CE3. Their immunoglobulins classes belong to IgG2a, IgG3 and IgM respectively. Four McAbs of IgG3 globulins were found to react with protein A for the Fc region. Whereas two McAbs of IgM classes and IgG2a subgroup were nonreactive or weak reactivity. Four McAbs of IgG3 can be used in detecting TmRSV by Staphylococcal Protein A—Enzyme Linked Immunosorbent Assay (SPA—ELISA) in this test. All six McAbs can trap many particles of tomato ringspot virus isolate from Canada by Immuno Electron microscope (IEM), three of them trapped the particles of TmRSV isolate from West Germany; and the rest of McAbs didn't do. This showed that there is a common antigenic determinant at least in the coat proteins of both isolates.

A good result was obtained when the monoclonal antibodies were tested for detecting TmRSV by IEM and SPA—ELISA. The two methods are reliable, simple, fast. But both have individual advantages of saving antibodies and detecting for large number of samples. This study should prove to be a feasible detection technique for import quarantine services.

Cloning and expression of P1, HC, NIa, NIb and partial Vpg genes of turnip mosaic virus Hangzhou isolate 1

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Turnip mosaic virus(TuMV) belonging to the potyvirus group causes many losses in agricultural, pasture, horticultural and ornamental crops and is transmitted by many aphid species. The HC(helper component) protein of potyvirus has so far been implicated in two

major functions, it is required for aphid transmission, and acts as proteinase for autolytic cleavage of the HC—P1 junction. The turnip mosaic virus Hangzhou isolate 1 (TuMV H1) which infected *Brassica nepella* more seriously than other TuMV isolates, was isolated in Hangzhou. The genome of the TuMV H1 was ssRNA with the length of approximately 9. 7kb. For study of the functions of TuMV H1 HC, two primers, primer1 of 5' — GGGGATCCATGAGCACGCGTTGGCGCCAACT — 3' and primer2 of 5' — CCCCCATG-GTTATCCAACGCGGTAGTGTTTC—3' were designed. The purified TuMV H1 RNA was reverse transcribed with the primer2 and the synthesized first—stranded cDNA of TuMV H1 RNA was used as a template. With the primer1 and primer2, a 1. 3kb of fragment was amplified by PCR, the product was purified by Wizard PCR Preps system from Promega, the amplified product was filled with klenow enzyme and cloned into Sma I site of pUC19. A recombinant , 4. 0kb in length, containing the 1. 3kb of fragment of HC gene was named as pUCHC. The fragment insertion orientation has been determined by analysis of the restriction sites. The recombinant pUCHC was digested with SacI and SalI, and cloned into SacI and SalI sites of the expression vector pGEMEX— 1. The recombination vector with 5. 3kb in length was named as pGEMHC. A fusion protein with MW 77kDa containing HC protein has been expressed with IPTG induction in E. coli JM109(DE3) . To obtain a large fragment of TuMV H1, Primer3 of 5' — ATGTCGACATCGCTGCGCAGATTGC — 3' from the downstream of Nlb(nuclear inclusion protein b) gene was designed. The purified TuMV H1 ssRNA was reverse transcribed with the primer3. The double—strand cDNA was synthesized and cloned into SmaI site of pUC18. The obtained four recombinants contained 0. 4, 1. 1, 1. 5 and 2. 6kb of cDNA fragment, respectively. The homology among these fragments was determined by Southern blot, and the results proved that the fragments were synthesized from the primer3. By using the 2. 6kb fragment as a template, when adding primer3 and primer4 of 5'—GGGGATCCATGACCCAGCAGATCGGTGGATGTTC—3' in PCR, a fragment of 1. 3kb which was equal to Nlb gene in length was produced, and when using the primer5 of 5'—GGATATCATGAGTAACTCCATGTTCAGAGGG—3' and primer6 of 5'—GGGGATCTTATTGTGCGTAGACTGCCGTGCT—3', the 0. 7kb of Nla(nuclear inclusion protein a) gene fragment has been produced. According to TuMV sequence(Olivier, et al. , 1992), the recombinant included 2. 6kb of insertion fragment containing 1. 3kb of Nlb and 0. 7kb of Nla genes except for approximately 0. 6kb of partial Vpg gene(the entire Vpg gene is 0. 73kb in length). The 2. 6kb recombinant is being sequenced. TuMV RNA encode a polyprotein. This polyprotein is proteolytically processed into at least eight mature proteins by three virus encoded proteinases, among which one is N—terminal protein P1, the P1 protein may be involved in specific virus— host interactions and has relationship with cell—to—cell movement proteins of viruses. But its functions are not clear. Using the TuMV H1 RNA as a template, two primers, primer7 of 5'—AATTCATGGCAGCAGTCACATTTG—3' and primer8 of 5' — GGGGATCCTTAAAAATGTACGATCTTAAGACT — 3', were designed,

the 1. 1kb of P1 fragment was synthesized by RT—PCR, and it was cloned into EcoRI and BamHI sites of pUC19. The recombinant with 3. 8kb containing a 1. 1kb insertion fragment termed pUCP1 was obtained. After the P1 gene was digested with EcoRI and BamHI from pUCP1, and it was cloned into EcoRI and BamHI sites of pGEMEX—1. A recombination expression vector was obtained and named pGEMP1, 5. 1kb in length. Using the vector, a approximately 70KDa of fusion protein including the P1 protein was expressed by IPTG induction in E. coli JM109 (DE3) bacterial strain. It was found that there were some differences between TuMV H1 and TuMV(Olivier, et al. , 1992) in P1, HC, Vpg, NIa, NIb regions by restriction enzyme analysis. The above results provide possibility for study of functions of the genes and host—virus—gene interaction from TuMVH1.

Progress of studying on beet necrotic yellow vein virus (BNYVV) transmitted by *Polymyxa betae*

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Rhizomania disease of sugarbeet caused by BNYVV has developed quickly and widely in major sugarbeet area of China. It decreases the yield and sugar content and harms the breeding of beet.

BNYVV is a member of furoviruses with multicomponent genome. We started to study rhizomania disease from early 1980s and the work is still continuing. The progress of study on diagnosis of causal agent, application of techniques of genetic engineering for constructing transgenic sugarbeet resistant to BNYVV and vector Polymyxa betae are being introduced respectively.

1. Techniques for detection of BNYVV

(1)A practical procedure for purifying BNYVV with good purity and yield was developed from a method described by Tamada. It ensured the antiserum preparation and RNA extraction for further work.

(2)BNYVV—NM (Inner Mongolia isolate) antisera with the titer of 1:1024 in microprecipiting test were prepared by both of the routine and the microdosage immunization schedules. The results of indirect ELISA test confirmed the occurrence of rhizomania in Xinjiang, Ningxia, Jiling and Heilongjiang provinces. DAS — ELISA kit of BNYVV with horseradish peroxidase conjugate was prepared.