식물조직배양학회지 제21권 제6호
Korean J. Plant Tissue Culture
Vol. 21, No. 6, 353~356 (1994)

Expression of Antibody Genes Specific for Human Hepatitis-B Virus in Transgenic Tobacco Plants

Seok Yoon KWON[1], Shin Je KIM[1,2], Hyo Jeong HONG[1], Moon Hi HAN[1], Chang Ho CHUNG[2], Ho Sul LEE[2], and Kyung Hee PAEK*[,1]

[1]Genetic Engineering Research Institute, KIST, Taejon, 305-606: and
[2]Kumho Chemical Laboratories, Korea Kumho Petrochemical Co., LTD., Taejon, 305-600. *Corresponding author.

형질전환된 담배에서 사람 B형 간염바이러스 항체 유전자의 발현

권석윤[1] · 김신제[1,2] · 홍효정[1] · 한문희[1] · 정창호[2] · 이호설[2] · 백경희*[,1]

[1]한국과학기술연구소 유전공학연구소, [2]금호석유화학(주)연구소

Chimeric kappa-chain and gamma-chain cDNA clones (pCKS2 and pCHS2) of a monoclonal antibody specific for pre-S2 surface antigen of human hepatitis-B virus were ligated into *Xba*I site of plant expression vector, pBKS-1. Plasmid DNA containing each of the chimeric gene were then mobilized from *E. coli* to *Agrobacterium tumefaciens* strain LBA4404. The chimeric antibody genes were then introduced into tobacco by Ti plasmid-mediated transformation. The putative transformants were selected on medium containing kanamycin sulfate. Shoots that formed on shoot induction medium were analyzed by Western blot analysis for the expression of kappa-chain or gamma-chain genes. The Western blot analyses clearly showed that the introduced genes were stably expressed in transgenic plants.

Key words: *Agrobacterium* **Ti plasmid-mediated transformation, gamma chain, immunoglobulin G, kappa chain**

Expression of foreign genes in plants has been achieved by recombinant DNA technology and *Agrobacterium* Ti plasmid-mediated plant transformation method. A variety of bioactive compounds such as human serum albumin (Sijmons et al., 1990), mouse metallothionin (Maiti et al., 1989), and monellin (Penarrubia et al., 1992) have been expressed in transgenic plants.

Plants that produce monoclonal antibodies are of interest, because in addition to synthesis of two gene products (i.e., the heavy and light chains), the two polypeptides need to be assembled correctly in order to become a functional antibody. Successful expression of antibody genes in plants was recently reported (Hiatt et al., 1989: Owen et al., 1992). Kappa chain gene and gamma chain gene were introduced independently into plants and assembled antibody proteins were obtained by sexual crossing of kappa- or gamma-chain gene expressing plants. Or single-chain gene of Fv protein was introduced into plant and expression of antibody genes was confirmed (Boston et al., 1991: During et al., 1990: Fontes et al., 1991).

Antibody production in plants could be applied to growth regulation of plant (Owen et al., 1992) and resistance to plant pathogen (Tavladoraki et al., 1993). In this study, as a first step of functional antibody production, we introduced the kappa- and gamma-chain gene (850 bp and 1,590 bp, respectively) of human hepatitis-B virus antibody (Hong et al., 1992) into tobacco and analyzed the production of kappa- and gamma-chain production in T_0 generation.

MATERIALS AND METHODS

Bacterial Strain and Plasmids

E. coli strain HB101 and *Agrobacterium tumefaciens* LBA4404 were used as bacterial host. pCHS-2 and pCKS-2 (Hong et al., 1992) had heavy chain gene and kappa chain gene in pBluescript, respectively. pBKS-1 was plant expression

vector that contained NPTII gene for plant selection marker, CaMV 35S promoter, and NOS terminator (Song and Hong, 1991).

Plasmid Construction

To transfer the heavy- and kappa-chain gene into expression vector, pCHS-2 and pCKS-2 were digested by *Sal*I, *Sal*I to *Xba*I adaptors (5'TCGATCTAGAAA and 3'AGATCTTT: Figure 1) were added, and *Xba*I inserts (0.9 kb and 1.5 kb, respectively) were obtained. To confirm the orientation and size of the inserts, *Bam*HI, *Hin*dIII, *Eco*RI and *Sac*I restriction enzyme digestion analyses were performed. Correctly oriented pCKS-2/BKS-1 or pCHS-2/BKS-1 were transfered to *Agrobacteium* by direct transfer method (An, 1987). Transformants from *Agrobacterium* were confirmed by *Eco*RI digestion analysis.

Plant Transformation

Plant transformation was performed by leaf disk cocultivation method (Horsch et al., 1985). That is, *A. tumefaciens* LBA4404 carrying pCKS-2/BKS-1 or pCHS-2/BKS-1 was grown o/n in YEP medium at 28°C and resuspended in MS medium (Murashige and Skoog, 1962). The tobacco explants were then cocultivated with *Agrobacterium* for 48 h at 25°C. The explants were then washed in MS medium and placed on shoot induction medium (MS salt + BA 0.5 mg/l + 30% sucrose, pH 5.6) containing kanamycin sulfate (200 mg/L) and carbenicillin (250 mg/L). Regenerants selected from the MS medium containing kanamycin were then transplanted to potting soil. The leaves of fully grown tobacco plants were used for further analysis.

Western Blot Analysis

AP-conjugated anti-human antibody (Promega Co.) were used for Western blotting analysis. Proteins extracted from transformed tobacco plants were separated on 10% SDS-PAGE and either stained with Coomassie brilliant blue or transferred to nitrocellulose paper (Hoefer Scientific Inst.) in TAE buffer. Blocking and antibody treatment were performed essentially according to the manufacturer's instruction.

RESULTS AND DISCUSSION

Construction of Kappa- or Gamma-Chain Gene in Plant Expression Vector

Figure 1 shows the scheme of plasmid construction of pCKS-2/BKS-1 or pCHS-2/BKS-1. To confirm the orientation and size of the cDNA inserts in pBKS-1, restriction enztme digestion analysis were carried out with *Bam*HI, *Eco*RI, *Hin*dIII, *Sac*I and *Eco*RI/*Hin*dIII. Upon *Bam*HI digestion, 0.9 kb or 1.5 kb insert could be identified from pCKS-2/BKS-1 or pCHS-2/BKS-1, respectively (Figure 2A, B). Recombinant plasmid DNA containing correctly oriented DNA insert against CaMV35S promoter were used for *A. tumefaciens* transformation. Transformants were confirmed by *Eco*RI digestion analysis of plasmid DNA from *A. tumefacien* (Figure 3). In this case, 1.1 kb restriction fragment from pCKS-2/BKS-1 and 1.8 kb from pCHS-2/BKS-1 could be identified. With *Eco*RI digestion, NOS terminator in addition to the cDNA insert should be included (Figure 1).

Analysis of Tobacco Plants Transformed with Either pCKS-2 or pCHS-2

Putative transformants were selected from medium containing kanamycin sulfate. Western blot analysis was then carried out to find out whether the cDNA clones of kappa- or gamma-chain could be expressed in these transgenic tobacco plants. When AP-conjugate anti-human immunoglobulin G (IgG) was used as antibody, approximately 30 kDa from plant transformed with pCKS-2/BKS-1 (lane 1, Figure 4B) and 60 kDa polypeptide from plant with pCHS-2/BKS-1 (lane 2, Figure 4A) could be identified. These polypeptide sizes are about same size when expressed in *E. coli* (Hong et al., 1992). This result means that chimeric kappa-chain and gamma-chain genes of antibody against pre-S2 surface antigen of human hepatitis-B virus were well expressed at least in these transgenic tobacco plants.

Thus we confirmed cDNA clones of kappa- or gamma chain were well expressed in transgenic tobacco system. In the future, it will be interesting to prove whether these chains could assemble and make functional IgG in these plants. We are currently in the middle of analysing the progeny of sexual-crossed transgenic plants expressing either kappa chain or gamma chain gene.

Plant antibody technology is still quite new. However, it offers enormous potential in 'mix-and-match' antibody

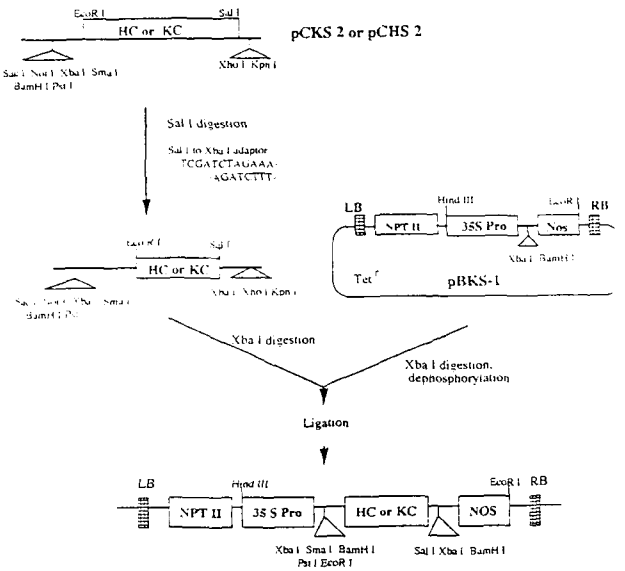

Figure 1. Schematic diagram showing cloning of cDNA coding for kappa chain or gamma chain specific for pre-S2 antigen of hepatitis B virus into pBKS-1.

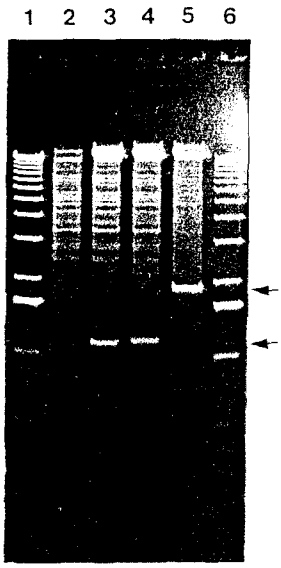

Figure 3. Restriction digestion patterns of plasmid DNA from *Agrobacterium tumefaciens*. Lanes 1 and 6, 1 kb ladder: 2, *A. tumefaciens* LBA4404, *Eco*RI: 3 and 4, LBA4404 transformed with pCKS-2/BKS-1, *Eco*RI: 5, LBA4404 transformed with pCHS-2/BKS-1, *Eco*RI. The upper arrow indicates *Eco*RI fragment with gamma chain, the lower arrow kappa chain.

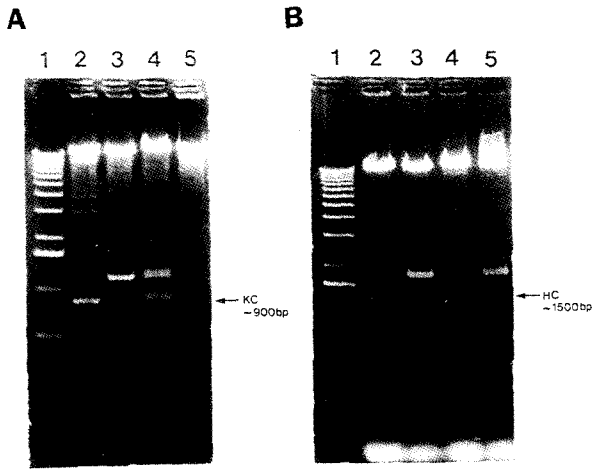

Figure 2. Restriction digestion analysis of pCKS-2/BKS-1 (A) and pCHS-2/BKS-1 (B). (A) Lane 1, 1 kb ladder: 2, *Bam*HI: 3, *Eco*RI: 4, *Eco*RI/*Hin*dIII: 5, *Sac*I. (B) Lane 1, 1 kb ladder: 2, *Bam*HI: 3, *Eco*RI: 4, *Hin*dIII: 5, *Eco*RI/*Hin*dIII. KC and HC designate cDNA inserts of kappa chain and gamma chain, respectively.

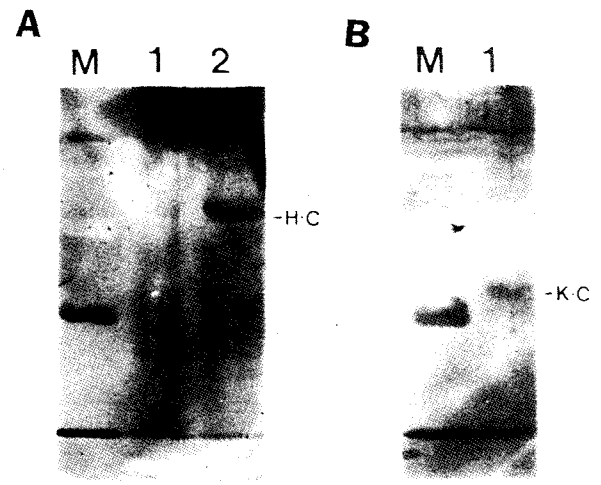

Figure 4. Western blot analysis of kappa chain and heavy chain protein from T0 transgenic tobacco plants. M: protein marker. (A) transgenic plants producing gamma chain (B) kappa chain producing transgenic plants. The arrows indicate the positions of kappa chain (KC) or gamma chain (HC). Protein sample prepared from nontransformed tobacco plant as negative control is shown on the lane 1 of (A).

engineering, and the construction of multimeric immunoglobulin complexes may be feasible relatively easily. Furthermore, since there is an enduring interest in using antibodies for therapeutic purposes, possibility of agricultural production and distribution offers a means of obtaining large quantities of antibodies at a relatively low cost.

적 요

사람 B형 간염 바이러스의 pre-S2 표면항원에 결합하는 키메라 항체 유전자(카파 및 감마사슬의 cDNA클론)를 식물체에서 발현시키기 위해 식물체 발현벡터인 pBKS-1에 *Xba*I 자리를 이용하여 클로닝하였다. 이들 유전자를 포함하는 대장균의 플라스미드 핵산을 추출하여 아그로박테리움에 형질전환 시켰다. 다음 담배의 조직절편과 아그로박테리움을 공동배양함으로써 담배의 형질 전환을 시도하였다. 카나마이신이 포함된 신초유기배지에서 나온 신초를 시료로 하여 Western blot을 실시함으로써 이들 유전자가 형질전환

담배에서 안정하게 발현됨을 확인하였다.

ACKNOWLEDGEMENTS - This work was supported by a grant (N81040) from Korea Ministry of Science and Technology to K.-H. Paek.

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(Received November 7, 1994)