Plant Pathology Bulletin 17: 279-287, 2008

Molecular cloning of sigma factor gene of phytoplasma associated with peanut witches' broom

Shau-Kwaun Chen [1], Pei-Wen Chu [1], Kuo-Chieh Ho [2], Wuu-Yang Chen [1] and Chan-Pin Lin [1, 3]

[1] Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan 106, R.O.C.

[2] Department of Life Science, National Taiwan University, Taipei, Taiwan 106, R.O.C.

[3] Corresponding author, E-mail: cplin@ntu.edu.tw; Fax: +886-2-2366-1980

Accepted for publication: May 28, 2008

ABSTRACT

Chen, S. K., Chu, P. W., Ho, K. C., Chen, W. Y., and Lin, C. P. 2008. Molecular cloning of sigma factor gene of phytoplasma associated with peanut witches' broom. Plant Pathol. Bull. 17: 279-287.

To elucidate the molecular biology of phytoplasmas, a gene encoding the primary sigma factor of the phytoplasma associated with peanut witches' broom (PnWB) was cloned and sequenced. One complete open reading frame (ORF) was determined on the insert of the selected recombinants. The ORF encodes a polypeptide contains -10 recognition and binding region, -35 recognition and binding region, core binding region and *rpoD* box. The gene organization and the nucleotide sequence in conserved region of the ORF are similar to other prokaryotic *rpoD* homologous sigma factor genes. According to the nucleic acid and amino acid sequence analyses, along with the results of Northern hybridization analyses, this gene was identified as a putative sigma factor gene. Based on the results of Southern hybridization analyses, it suggests that three *rpoD* homologous sigma factor genes may exist in PnWB phytoplasma.

Key words: *Mollicutes*, *rpoD* gene, RNA polymerase, PCR-based cloning

INTRODUCTION

Phytoplasmas, a group of prokaryotes lacking cell wall, are associated with a variety of plant diseases in several hundreds of plant species [1, 18, 25]. Understanding the physiology, biochemistry and molecular biology of phytoplasmas is limited because of their resistance to culturing under axenic conditions [14], while procedures available for purification of phytoplasmas from disease plants are tedious and unsatisfactory [3, 10] due to the inevitable contamination of plant component. However, molecular methods had been applied to improve the methodology of the studies of phytoplasmas since late 1980s, especially phytoplasma DNA can be purified and isolated from plant DNA by CsCl equilibrium buoyant density centrifugation based on its extreme AT bias of codon usage [12, 24]. Consequently, the molecular biology of phytoplasmas can now be studied, and it seems to be the best way to approach and characterize these mysterious prokaryotes.

Sigma factor is a key subunit of prokaryotic RNA polymerase (RNAP), which is an essential enzyme in prokaryotic genes transcription. Associating with core subunits of RNA polymerase to form a holoenzyme, sigma

factors function as recognizing and binding to -10 and -35 consensus sequences in the promoter region to facilitate the initiation of gene transcription. Most bacterial species synthesize several different sigma factors, which direct the RNA polymerase holoenzyme to distinct classes of promoters with a different consensus sequence [30]. It reveals that the initiation of transcription is the most prominent step in gene regulation.

Based on sequence similarity, bacterial sigma factors could be roughly divided into two broad classes: primary sigma factors σ^{70} and related sigma factors, which initiate transcription of the housekeeping genes; and σ^{54} and other alternative sigma factors, which recognize promoters with different sequences for genes corresponding to growth under different physiological conditions [7]. The σ^{70} family has been divided broadly into four phylogenetic groups on the basis of gene structure and function [20, 21, 22]. Group 1 consists of the essential primary σ factors, each of which is closely related to σ^{70} of *E. coli*. Group 2 proteins are closely related to the primary σ factors but are dispensable for bacterial cell growth. Group 3 σ factors are more distantly related to σ^{70} and usually activate regulons in response to specific signal, such as a developmental checkpoint or heat shock. The group 3 σ factors can be further divided into several clusters of functionally related proteins with roles in sporulation, flagella biosynthesis, or the heat-shock response, for example. Finally, group 4 accommodates the numerically largest, but highly diverged extracytoplasmic function (ECF) subfamily, most members of which respond to signals from the extracytoplasmic environment, such as the presence of misfolded proteins in the periplasmic space. Whereas most bacteria have a single group 1 primary σ factor, the number of other group members varies widely, reflecting the different physiological and developmental characteristics of the various organisms [20, 21, 22]. The number of σ^{70} family members may vary from 1 (*Mycoplasma* sp.) to over 60 (*Streptomyces coelicolor*) [6]. Most bacterial genomes encode multiple σ factors that are required for complex cellular processes such as stress response, morphogenesis and virulence [28]. In general, organisms with more varied lifestyles contain more sigmas. This is expected because organisms that encounter varied environments need to adjust their metabolism and respond to many stresses, which requires a large repertoire of regulatory mechanisms [6].

Four regions of high conservation are observed after aligning the amino acid sequences of all known σ^{70} genes (*sigA* and *rpoD*) [17]. Several of the further divided subregions are suggested to be functional domains, including core binding, recognition and binding of -10 and -35 consensus sequences and *rpoD* box [4, 29]. Structurally, the four major regions of σ^{70} family factors have the highest levels of conservation in region 2 and 4. Subregions within region 2 are involved in promoter melting (region 2.3) and -10 sequence recognition (region 2.4). Region 4.2 is involved in -35 recognition [6, 11, 19, 22]. Such high conservation of gene structure and nucleic acid sequences between σ^{70} genes make PCR-based strategy a preferable way for gene cloning. In this study, we describe a gene encoding the primary sigma factor of the phytoplasma associated with peanut witches' broom (PnWB) was cloned and sequnced using PCR-based strategy. Further study of sigma factor could increase our understandings to how phytoplasmas transcribe their gene and how the expression of the genes are regulated.

MATERIALS AND METHODS

Source of phytoplasmas

PnWB phytoplasma-affected periwinkle (*Catharanthus roseus* (L.) G. Don) oringinally obtained by transmission through dodder (*Cuscuta australis* R. Broom) was provided by I. L. Yang (Taiwan Agricultural Research Institute, Wufeng, Taiwan) and was maintained and propagated in periwinkle by side grafting [13, 26].

Purification of phytoplasma DNA and genomic library construction

Total DNA was isolated from healthy and phytoplasma-affected periwinkle plant tissues according to the method described by Ko and Lin [13]. To separate phytoplasma DNA from total DNA, a CsCl-bisbenzimide density-gradient centrifugation method was used [13]. Approximately 250 ng purified PnWB phytoplasma DNA was digested with the restriction enzyme *Eco*RI and ligated to 2 μg *Eco*RI-cleaved calf-intestine-phosphatase-dephosphorylated lambda ZapII cloning vector, and then packaged with Gigapack II Gold packaging extract

according to the manufacturer's instructions (Stratagene, CA).

PCR amplification of PnWB phytoplasma *rpoD* gene fragment

A polymerase chain reaction (PCR)-based strategy was used to amplify the sigma factor gene *rpoD* of PnWB phytoplasma. The nucleotide sequences of *sigA* (encoding a major sigma factor, σ^A protein) of *Bacillus subtilis* (GenBank accession no. M84964), and *rpoD* genes of *Escherichia coli* (AP009048), *Lactococcus lactis* (AM406671), *Staphylococcus aureus* (CP000703), *Clostridium acetobutylicum* (M74569), *Listeria monocytogenes* (AE017262) and *Mycoplasma genitalium* (L43967) were aligned with program CLUSTAL [8]. A pair of degenerate primer, SF-f1 (5'-GATTTRATYCADGARGGWAA-3') and SF-r1 (5'-GCTTTKKYTTCDATTTG-3'), was designed based on the sequences of *rpoD* box and -35 recognition and binding region to amplify PnWB phytoplasma *rpoD* gene fragment. The PCR product is predicted to be an approximate 600 bp fragment.

Total DNA and purified phytoplasma DNA were both used as template for PCR. The PCR was performed in a 50 μl of reaction mixture containing 100 ng of template DNA, 3 μl of each primer (20 μM), 250 μM of dNTP mixture, 5 μl of 10x *Taq* reaction buffer (500 mM KCl, 15 mM $MgCl_2$, 100 mM Tris-HCl, pH 9.0 at 25℃, 1% Triton X-100), and 2.5 units of *Taq* DNA polymerase (Promega Corporation, WI). Thirty-five PCR cycles were conducted in a DNA Thermal Cycler 480 (Perkin Elmer, CA) with the following parameters: denaturation at 94℃ for 30s, annealing at 42℃ for 60s and extension at 72℃ for 30s. PCR products were purified, cloned directly into a TA cloning vector (pCR II) (Invitrogen Corporation, CA) according to the manufacturer's instructions and then sequenced.

Genomic library screening

The PCR-amplified 600 bp *rpoD* gene fragments were labeled with digoxigenin-11-dUTP using DIG DNA labeling and detection kit (Boehringer Mannheim GmbH, Germany) according to the manufacturer's instruction. The digoxigenin-labeled nucleic acid probe was then used to screen the phytoplasma genomic library. Overnight cultures of *Escherichia coli* XL1 Blue grown in LB supplemented with 0.2% maltose and 10 mM $MgSO_4$ were harvested and infected with the recombinant phage (10^5 pfu per plate of 150 mm in diameter) at 37℃ for 20 min. Infected cells were then plated on NZY plates (0.5% NaCl, 0.2% $MgSO_4$, 1.5% Bacto agar, pH 7.5) and incubated at 37℃ overnight. Plaques were lifted with MagnaGraph nylon filter (Micron Separation Inc., MA) and the DNA was immobilized on the filter according to manufacturer's manual. Filters were hybridized with digoxigenin-labeled probe following the manufacturer's instructions. Plaques giving positive signals were isolated and subjected to secondary and tertiary screenings to ensure plaque purity.

In vivo excision of the recombinant pBluescript SK(-) phagemid containing the cloned gene was performed using the ExAssist/ SOLR system (Stratagene).

Sequence analysis of the *rpoD* gene

Nucleotide sequences were determined by the dideoxy chain-termination method [23] using the Sequenase version 2.0 DNA sequencing kit (US Biochemical, OH). The entire nucleotide sequence of the selected recombinant plasmid pPSF3 was read on both strands. Homologous sequences were searched in the GenBank databases using program BLAST. Furthermore, the nucleotide sequence was analyzed, including finding ORF, codon usage analysis and secondary structure prediction with the computer program Lasergene (DNASTAR Inc., WI). Multiple alignment of the nucleotide sequence of *rpoD* genes was performed with the computer program CLUSTAL [8].

Southern and Northern hybridization

For Southern hybridiaztion, 3 μg total DNA from healthy periwinkle plant and PnWB phytoplasma-affected periwinkle plant were digested with *Eco*RI, *Bam*HI and *Xba*I (Boehringer Mannheim GmbH), and then seperated in a 0.8% agarose gel. Nucleic acid probe used in Southern hybridization is the same as that used in genomic library screening. Hybridization was performed under low and high stringency at 55℃ and 68℃, respectively [2, 13]. For Northern hybridization, 30 μg total RNA of healthy and PnWB phytoplasma-affected periwinkle plants was isolated according to the method by Yeh *et al.* [31], and hybridization was conducted at 55℃ using the

digoxigenin-labeled 1.8-kb insert of selected recombinant plasmid pPSF3.

RESULTS AND DISCUSSION

Amplification of a putative *rpoD* fragment of PnWB phytoplasma

A specific 0.6 kb DNA fragment was amplified from either total DNA of PnWB phytoplasma-affected periwinkle plants or phytoplasma DNA purified with CsCl-bisbenzimide density-gradient centrifugation method (Fig. 1, lanes 2 and 3) with primers SF-f1 and SF-r1, while no PCR product was obtained using DNA template prepared from healthy periwinkle plant. The amplified DNA fragment was sequenced and aligned to other prokaryotic *rpoD* genes. The sequences of amplified fragment showed at least 80% nucleotide sequences identity with known prokaryotic *rpoD* in the GenBank database. Especially in the highly conserved regions, including *rpoD* box and -10 and -35 sequences recognition and binding region, the amplified DNA fragment share extremely high identity with the nucleic acid sequences of the *rpoD* genes compared. It strongly suggests that it should be a gene fragment of phytoplasma *rpoD* gene.

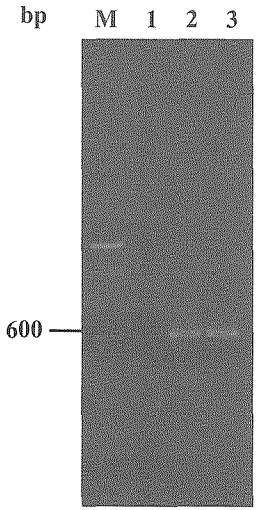

Fig. 1. Amplified polymerase chain reaction (PCR)-product of total DNA of healthy periwinkle plant and periwinkle plant affected with PnWB-phytoplasma using primers SF-f1 and SF-r1. DNA template were prepared from healthy periwinkle plant (lane 1), periwinkle plant affected with PnWB-phytoplasma (lane 2), periwinkle plant affected with PnWB-phytoplasma and subjected to CsCl-bisbenzimide centrifugation (lane 3). M, 100 bp ladder as molecular weight standards. Size (in bp) of PCR product is shown on the left.

Sequence analyses of the *rpoD*

By screening recombinant phages of the PnWB phytoplasma genomic library, six positive clones were selected, *in vivo* excised and then confirmed by Southern hybridization analyses. All 6 clones contained a 1.8 kb insert and shared the same nucleotide sequences. One of the recombinant plasmids was selected and designed as pPSF3 for further studies. The complete nucleotide sequence of the 1,768 bp insert DNA of pPSF3 was determined and submitted to the GenBank database with an accession number AF160964. Based on the universal codon usage, one putative ORF was identified. The ORF contains 1,377 bp (nts 138-1,514), started from the ATG initiation codon and stopped at the translation termination codon TAA. The coding region of the gene encodes a polypeptide of 458 a. a. with a calculated MW of 54.8 KD. A putative Shine-Dalgano sequence AGGAGG (nts 128-133), which is complementary to the 3' end sequence of 16S rRNA of phytoplasmas, was found, but no consensus -10 and -35 sequences were identified.

The base composition of the gene is 41.06 mol% of A, 12.12 mol% of C, 16.65 mol% of G, and 30.17 mol% of T. A low G+C content (28.77%) of the gene, along with a preferential use of A- and T- rich codons, and the high frequency of the use of A or T residues at the 5'-end (1st base) (64.85%) and 3'-end (3rd base) (72.05%) of codons were observed, which are similar to the features of other phytoplasmas[12, 15, 24]. Fifty AAA lysine codons appeared in the gene, which made it the most frequent codon. In the *rpoD*, 12 tryptophan residues are encoded by UGG. The result agrees with that of a previous study on the ribosomal protein genes for *Oenothera* phytoplasma[15]. In the class *Mollicutes*, mycoplasmas and spiroplasmas both utilize UGG and UGA triplets as tryptophan codons[9, 16], but *Acholeplasma laidlawii* utilizes UGG only[16, 27].

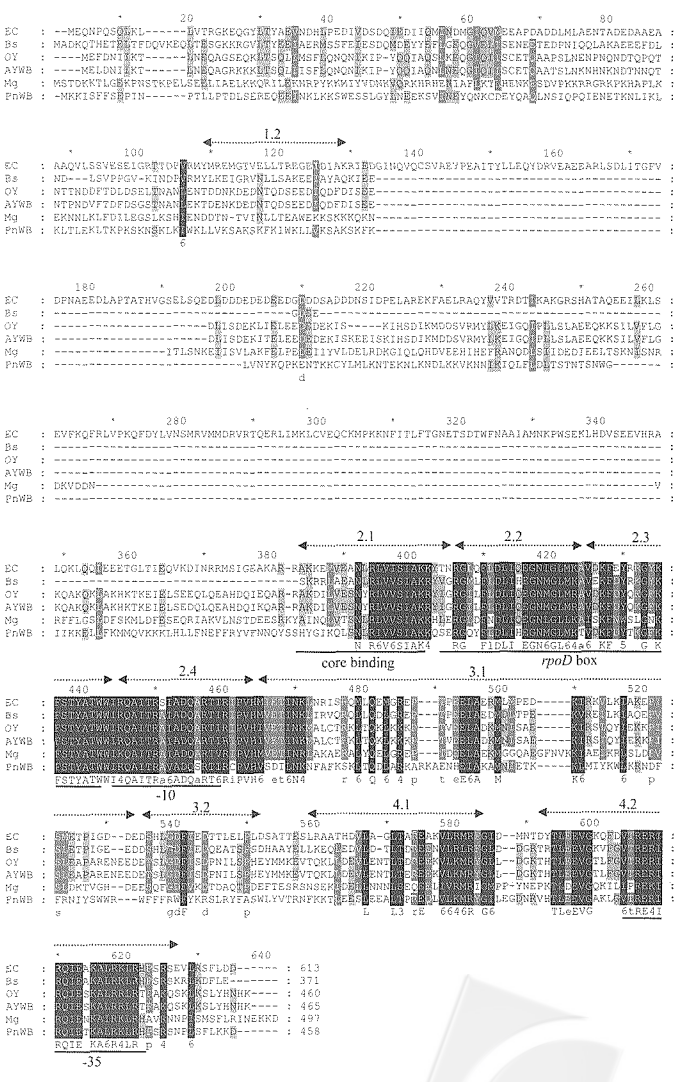

Fig. 2. Multiple amino acid sequence alignment of the putative sigma factor from recombinant plasmid pPSF3 (PnWB) (GenBank accession no. AF160964) and *sigA* gene in *Bacillus subtilis* (Bs) (M84964), and *rpoD* gene in *Escherichia coli* (Ec) (AP009048), onion yellows phytoplasma (OY) (AP006628), aster yellows witches' broom phytoplasma (AYWB) (CP000061) and *Mycoplasma genitalium* (My) (L43967) conducted by CLUSTAL sequence analysis program. Residues shaded in black are conserved in those of all 6 species compared, residues shaded in dark grey are conserved in those of 5 species, and residues shaded in light grey are conserved in those of less than 4 species. Subregions are indicated and shown by arrowed dotted lines above the sequences and conserved regions are underlined.

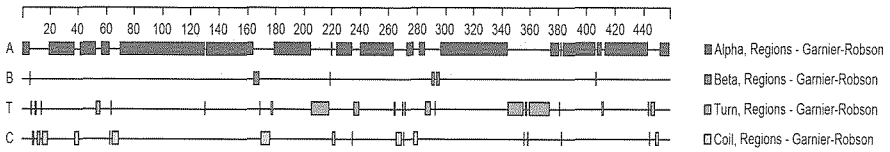

Fig. 3. Secondary structure of sigma factor of PnWB phytoplasma predicted by Garnier-Robson method. A: α helix, B: β sheet, T: β turns, C: coiled region.

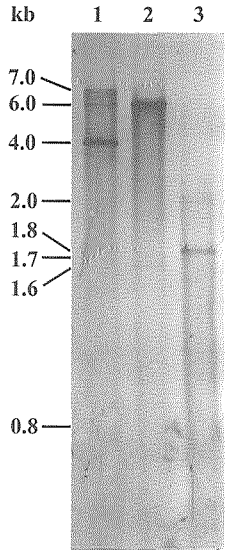

Fig. 4. Southern blot of total DNA prepared from diseased periwinkle plant affected with PnWB-phytoplasma digested with *Xba*I (lane 1), *Bam*HI (lane 2), *Eco*RI (lane 3). Sizes (in kb) of the hybridization signals are shown on the left.

Sequence similarities

The nucleotide sequence and the deduced amino acid sequence of the ORF were compared with other *rpoD* genes available in GenBank. This ORF shares 37% of nucleotide sequence identity and 28% of amino acid sequence identity with *rpoD* gene of *Mycoplasma genitalium* (GenBank accession no. L43967), while 36% of nucleotide sequence identity and 30% of amino acid sequence identity is observed comparing with *rpoD* gene of aster yellows witches' broom (AYWB) phytoplasma (CP000061, nts 125,358-126,755), and 43% of nucleotide sequence identity and 31% of amino acid sequence identity with *rpoD* gene of onion yellows (OY) phytoplasma (AP006628, nts 712,396-713,778). The result of multiple alignments of deduced amino acid sequences was shown as Fig. 2. It reveals that the gene organization and the nucleotide sequence in conserved region of this ORF is similar to other *rpoD* sigma factor genes. Other conserved regions including -10 recognition and binding region, -35 recognition and binding region, core binding region and *rpoD* box are also observed in the putative ORF. All of those regions are functional domains and responsible to major biological function of sigma factor, thus it suggests that this ORF encodes primary sigma factor of PnWB-phytoplasma.

The secondary structure of the putative sigma factor was also predicted based on deduced amino acid sequence using the method developed by Garnier *et al.*[5] (Fig. 3). Most of the *rpoD* genes could be divided into four regions. Region 1 is mainly composed of β sheet, while other regions are mainly composed of α helix. The putative secondary structure of phytoplasma *rpoD* gene is similar to other *rpoD* genes, except α helical region 1.

Southern hybridization

Total DNA of PnWB phytoplasma-affected periwinkle plant was digested with *Xba*I, *Bam*HI and *Eco*RI and hybridized with the DIG-labeled 600 bp *rpoD* gene fragment. The hybridization patterns were shown in Fig. 4. The probe hybridized with 3 bands at 4 kb, 6 kb and 7 kb of *Xba*I digest (lane 1), and 3 bands at 6 kb, 1.6 kb and 1.7 kb of *Bam*HI digest (lane 2), and 3 bands at 1.8 kb, 2.0 kb and 0.8 kb of *Eco*RI digest (lane 3). No positive signal was observed for the DNA prepared from healthy periwinkle plant in the Southern hybridization under both low and high stringencies (data not shown). These results

suggest that three *rpoD* homologues may exist in the genome of PnWB phytoplasma.

To compare the identity between different copies of *rpoD* in a prokaryote, four sequenced prokaryote genomes in GenBank were used for analysis. It was revealed that *E. coli* (AP009048) and *M. genitalium* (L43967) have only one copy of *rpoD* gene, while AYWB phytoplasma (CP000061) has 5 copies and OY phytoplasma (AP006628) has 4 copies of *rpoD* gene. Different copies of *rpoD* gene in AYWB phytoplasma share 24.1-85.3% of nucleotide sequence identity and 13.4-93.8% of amino acid sequence identity, while those of OY phytoplasma share 24.9-96.2% of nucleotide sequence identity and 12.1-96.6% of amino acid sequence identity. Since the results of Southern hybridization showed that there were one strong signal and two faint signals in all different enzyme digests, this may be due to the sequence differences between *rpoD* copies of PnWB phytoplasma.

Northern hybridization

The result of Northern hybridization was shown in Fig. 5. Total RNA purified from healthy periwinkle plant (lane 1) and from diseased periwinkle plant affected with PnWB phytoplasma (lane 2) were applied in the study. The relative quantities of 18S and 28S rRNA were used as a control for monitoring the amount of healthy and PnWB-affected periwinkle loaded for electrophoresis (data not shown). One 3.7 kb positive signal was detected for the RNA prepared from affected periwinkle plant, while no signal was observed for those prepared from healthy periwinkle plant. It suggests that this *rpoD* does expressed in PnWB phytoplasma.

Members of the σ^{70} family of sigma factors are components of the RNA polymerase holoenzyme that direct bacterial core RNA polymerase to specific promoter elements. The primary σ factor, which is essential for general transcription in exponentially growing cells, is reversibly associated with RNA polymerase and can be replaced by alternative σ factors that coordinately express genes involved in diverse functions, such as stress responses, morphological development and iron uptake. Here we shown the primary sigma factor gene, *rpoD*, exist in PnWB phytoplasma with three copies. Whether there is any alternative σ factor with different functions still need to be studied further.

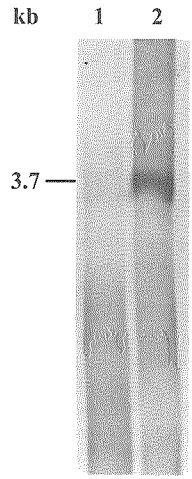

Fig. 5. Northern blot of total RNA prepared from healthy periwinkle plant (lane 1) and diseased periwinkle plant affected with PnWB-phytoplasma (lane 2). Hybridization was conducted at 55°C using the digoxigenin-labeled 1.8-kb insert of selected recombinant plasmid pPSF3. Size (in kb) of the hybridization signals is shown on the left.

LITERATURE CITED

1. Agrios, G. N. 2005. Plant diseases caused by mollicutes: phytoplasmas and spiroplasmas. Pages 687-703 *in*: Plant pathology, 5th ed. G. N. Agrios ed. Academic Press, San Diego, CA.
2. Chen, M. F., and Lin, C. P. 1997. DNA probes and PCR primers for the detection of a phytoplasma associated with peanut witches'-broom. Europ. J. Plant Pathol. 103:137-145.
3. Clark, M. F., Morton, A., and Buss, S. L. 1989. Preparation of mycoplasma immunogens from plants and a comparison of polyclonal and monoclonal antibodies against primula yellows MLO-associated antigens. Ann. Appl. Biol. 114:111-124.
4. Daniels, D., Zuber, P., and Losick, R. 1990. Two amino acids in an RNA polymerase sigma factor involved in the recognition of adjacent base pairs in the -10 region of a cognate promoter. Proc. Natl. Acad. Sci. USA 87:8075-8079.
5. Garnier, J., Osguthorpe, D. J., and Robson, R. 1978. Analysis of the accuracy and implications of a simple method for predicting the secondary structure of globular proteins. J. Mol. Biol. 120:97-120.

6. Gruber, T. M., and Gross, C. A. 2003. Multiple sigma subunits and the partitioning of bacterial transcription space. Annu. Rev. Microbiol. 57:441-466.
7. Helmann, J. D., and Chamberlin, M. J. 1988. Structure and function of bacterial sigma factors. Annu. Rev. Biochem. 57:839-872.
8. Higgins, D. G., and Sharp, P. M. 1988. CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. Gene 73:237-244.
9. Inamine, J. M., Ho, K. C., Loechel, S., and Hu, P. J. 1990. Evidence that UGA is read as a tryptophan codon rather than as a stop codon by *Mycoplasma pneumoniae*, *Mycoplasma genitalium*, and *Mycoplasma gallisepticum*. J. Bacteriol. 172:504-506.
10. Jiang, Y. P., and Chen, T. A. 1987. Purification of mycoplasma-like organisms from lettuce with aster yellows disease. Phytopathology 77:949-953.
11. Kazmierczak, M. J., Wiedmann, M., and Boor, K. J. 2005. Alternative sigma factors and their roles in bacterial virulence. Microbiol. Mol. Biol. Rev. 69: 527-543.
12. Kirkpatrick, B. C., Stenger, D. C., Morris, T. J., and Purcell, A. H. 1987. Cloning and detection of DNA from a nonculturable plant pathogenic mycoplasma-like organisms. Science 238:197-200.
13. Ko, H. C., and Lin, C. P. 1994. Development and application of cloned DNA probe for a mycoplasmalike organism associated with sweet potato witches' broom. Phytopathology 84:468-473.
14. Lee, I. M., and Davis, R. E. 1986. Prospects for *in vitro* culture of plant pathogenic mycoplasmalike organisms. Ann. Rev. Phytopathol. 24:339-354.
15. Lim, P. O., and Sears, B. B. 1991. DNA sequence of the ribosomal protein genes *rpl2* and *rpsl9* from a plant-pathogenic mycoplasma-like organism. FEMS Microbiol. Lett. 84:71-74.
16. Lim, P. O., and Sears, B. B. 1992. Evolutionary relationships of a plant-pathogenic mycoplasmalike organism and *Acholeplasma laidlawii* deduced from two ribosomal protein gene sequences. J. Bacteriol. 174:2606-2611.
17. Lonetto, M., Gribskov, M., and Gross, C. A. 1992. The σ^{70} family: sequence conservation and evolutionary relationships. J. Bacteriol. 174:3843-3849.
18. McCoy, R. E., Caudwell, A., Chang, C. J., Chen, T. A., Chiyowski, L. N., Cousin, M. T., Dale, J. L., de Leeuw, G. T. N., Golino, D. A., Hackett, K. J., Kirkpatrick, B. C., Marwitz, R., Petzold, H., Sinha, R. C. Sugiura, M., Whitcomb, R. F., Yang, I. L., Zhu, B. M., and Seemüller, E. 1989. Plant diseases associated with mycoplasma-like organisms, and Mycoplasmas of plants and Arthropods. R. F. Whitcomb and J. G. Tully, eds. Academic Press, San Diego, CA.
19. Nies, D. H. 2004. Incidence and function of sigma factors in *Ralstonia metallidurans* and other bacteria. Arch. Microbiol. 183:255-268.
20. Oguiza, J. A., Kiil, K., and Ussery, D. W. 2005. Extracytoplasmic function sigma factors in *Pseudomonas syringae*. Trends Micorbiol. 13:565-568.
21. Paget, M. S. B., and Helmann, J. D. 2003. The σ^{70} family of sigma factors. Genome Biology 4:203.
22. Rodrigue, S., Provvedi, R., Jacques, P. -É., Gaudreau, L., and Manganelli, R. 2006. The σ factors of *Mycobacterium tuberculosis*. FEMS Microbiol. Rev. 30:926-941.
23. Sanger, F., Nicklen, S., and Coulson, A. R. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74: 5463-5467.
24. Sears, S. E., Lim, P. O., Holland, N., Kirkpatrick, B. C., and Klomparens, K. L. 1989. Isolation and characterization of DNA from a mycoplasmalike organism. Mol. Plant-Microbe Interact. 2:175-180.
25. Seemüller, E., Marcone, C., Lauer, U., and Goschl, M. 1998. Current status of molecular classification of the phytoplasmas. J. Plant Pathol. 80:2-26.
26. Shen, W. C., and Lin, C. P. 1993. Production of monoclonal antibodies against a mycoplasmalike organism associated with sweet potato witches' broom. Phytopathology 83:671-675.
27. Tanaka, R., Andachi, Y., and Muto, A. 1989. Nucleotide sequence of tryptophan tRNA gene on *Acholeplasma laidlawii*. Nucleic Acids Res. 17:5842.
28. Wade, J. T., Roa, D. C., Grainger, D. C., Hurd, D., Busby, S. J. W., Struhl, K., and Nudler, E. 2006. Extensive functional overlap between σ factors in *Escherichia coli*. Nat. Struct. Mol. Biol. 13:806-814.
29. Waldburger, C., Gardella, T., Wong, R., and Susskind, M. M. 1990. Changes in conserved region 2 of *Escherichia coli* sigma 70 affecting promoter recognition. J. Mol. Biol. 215:267-276.
30. Wosten, M. M. S. M. 1998. Eubacterial sigma-factor. FEMS Microbiol. Rev. 22:127-150.
31. Yeh, K. W., Juang, R. H., and Su, J. C. 1991. A rapid and efficient method for RNA isolation from plants with high carbonhydrate content. Focus 13:102-103.

摘 要

陳紹寬[1]、朱佩文[1]、何國傑[2]、陳武揚[1]、林長平[1,3]. 2008. 花生簇葉病菌質體 sigma factor 基因之選殖與分析. 植病會刊 17: 279-287. ([1]台北市 國立台灣大學植物病理與微生物學系；[2]台北市 國立台灣大學生命科學系；[3]聯絡作者，電子郵件：cplin@ntu.edu.tw；傳眞：+886-2-2366-1980)

本實驗從事花生簇葉病菌質體 sigma factor 基因之選殖研究，實驗中以六種細菌之 *rpoD*, *sigA* 基因高保守性區域設計出一組 PCR 引子，以花生簇葉病菌質體 DNA 爲模板進行 PCR 反應而獲得一 600 bp 大小之 PCR 產物，並以此作爲核酸探針進行花生簇葉病菌質體基因庫之篩選，得到含 1.8 kb 嵌入片段之選殖株重組質體 pPSF3。對 pPSF3 之嵌入片段進行核苷酸序列分析可得到一個完整的 ORF (open reading frame)，且在其轉譯起始密碼 AUG 上游有互補於植物菌質體 16S rRNA 之 3' 端核酸序列即 Shine-Dalgarno 序列之存在，此爲可能之核糖體結合位置 (ribosomal binding site)。此 ORF 之核苷酸序列經推衍爲胺基酸序列後，發現其中有啓動子 -10 及 -35 辨識結合區域、中心酵素結合區域及 *rpoD* box，且基因大小及基因結構均與其他的 sigma factor 基因相似，故據此推斷其爲 sigma factor 基因。在南方氏雜合反應的結果可發現在花生簇葉病菌質體中可能有三個類似於其他眞細菌中 *rpoD* 基因之 sigma factor 基因。

關鍵詞：Mollicutes 綱、*rpoD* 基因、RNA 聚合酶、聚合酶連鎖反應選殖策略