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Sequence analysis of genes coding for the molecular chaperones GrpE, DnaK, and DnaJ from phytoplasma associated with peanut witches' broom

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ABSTRACT

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A PCR-based strategy was used for cloning *dnaK* and *dnaJ* genes of phytoplasma associated with peanut witches' broom (PnWB). Three nucleotide primers, KF1, KF2, KR1 were designed based on the conserved regions of eight bacterial *dnaK* genes. A 750-bp *dnaK* gene fragment was amplified using primer pair KF1/ KR1, and then KF2/ KR2 in semi-nested PCR. The 750 bp fragment and it's *Eco*RI-digested 200 bp fragment were used as probes for screening a genomic library of PnWB phytoplasma. Two recombinant plasmids, pBK1-1 (2.7 Kb) and pBSK3-1 (2.3 Kb), were obtained and sequenced. Based on the sequence analyses of the 750 bp *dnaK* fragment and the inserts of pBK1-1 and pBSK3-1, four open reading frames were identified to be arranged in the order of *hrcA*, *grpE*, *dnaK* and *dnaJ*. Chromosomal arrangement of these genes in PnWB phytoplasma is identical to those of aster yellows witches' broom phytoplasma, onion yellows phytoplasma and other bacteria phylogenetically related to phytoplasmas. This implies that the primitive bacterial molecular chaperone Hsp70 machine may exist in PnWB phytoplasma and its possible function when exposed to stress could be similar to those of other prokaryotes.

Keyword: *Mollicutes*, *dnaK* operon, semi-nested PCR

INTRODUCTION

Once a cell is exposed to stress such as heat shock, cold shock, osmotic shock and ultraviolet radiation, heat-shock genes will be induced and heat-shock proteins (HSPs) are transiently increased to facilitate the survival of most organisms [11, 25, 36]. The HSPs are divided into two broad classes of protein, molecular chaperones and proteases, which are highly conserved among prokaryotes and eukaryotes. [41] Based on both size and function, the HSPs are also classified into two groups of molecular chaperone nominated as the Hsp60 machinery (GroES/ EL) and the Hsp70 machinery (DnaK, DnaJ and GrpE), and two groups of protease, the Hsp90 and the Hsp20 families [4, 23]. Hsp60 and Hsp70 play essential roles in protein metabolism and protein translocation under both stress and non-stress conditions. In addition to this feature,

members of the Hsp70 family of molecular chaperones are involved in a wide variety of cellular processes [16], including protein folding and refolding [10, 29]. The gene organization *hrcA-grpE-dnaK-dnaJ* seems to be the basic and the most common arrangement of *dnaK* operon found in Gram-positive bacteria [2, 6, 14, 19, 28, 39]. DnaK activity is controlled by DnaJ and the nucleotide exchange factor GrpE [9, 26]. HrcA negatively regulates transcription of heat shock genes by binding to a conserved DNA element CIRCE (controlling inverted repeat of chaperon expression) located in the regulatory regions of the *dnaK* and *groE* operons [19, 26, 32]. CIRCE elements, with a consensus nucleotide sequence of TTAGCACTC-N_9-GAGTGCTAA, are usually present in the upstream of some heat shock genes such as *dnaK* and *groE* [38, 40, 42].

Phytoplasma, a group of prokaryotes lacking cell wall, are nonhelical mollicutes associated with diseases in several hundred plant species [1, 24, 34]. Understanding the physiology, biochemistry and molecular biology of phytoplasmas is limited because of their resistance to be cultured under axenic conditions [20]. In the last decade, molecular biology strategies have been brought into phytoplasmology more frequently. Phytoplasma DNA can be now successfully separated on its extreme AT bias of codon usage using a CsCl-bisbenzimide density gradient centrifugation method [17, 33]. Consequently, genetic information has become an important resource for phytoplasma study. In this work, homologous sequences of Hsp70 gene were collected from various bacteria to design primers for semi-nested polymerase chain reaction (PCR). The PCR-amplified fragments were cloned and used as probes to screen the genomic library of PnWB phytoplasma. Putative *dnaK* operon genes, *hrcA*, *grpE*, *dnaK*, and *dnaJ*, were thus cloned and identified in PnWB phytoplasma.

MATERIALS AND METHODS

Source of phytoplasmas

PnWB phytoplasma-affected periwinkle (*Catharanthus roseus* (L.) G. Don) originally 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 [18, 35].

Purification of phytoplasma DNA and genomic library construction

Total DNA was isolated from healthy and phytoplasma-affected periwinkle plants according to the method described by Ko and Lin [18]. To separate phytoplasma DNA, a CsCl-bisbenzimide density-gradient centrifugation method was applied [18]. 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 Zap II cloning vector (Stratagene, CA), and then packaged with Gigapack II Gold packaging extract (Stratagene) according to the manufacturer's instructions.

Polymerase chain reaction

The PnWB phytoplasma is phylogenically related to Gram-positive bacteria and the nucleotide sequence of *dnaK* is highly conserved among most eubacteria. The nucleotide sequences and deduced amino acid sequences of seven *dnaK* gene of four Gram-positive bacteria, *Bacillus subtilis* (GenBank accession no. M84964), *Lactococcus lactis* (AM406671), *Streptococcus pneumoniae* (U84387), *Clostridium acetobutylicum* (M74569), one Gram-negative bacteria *Escherichia coli* (AP009048), and three mollicutes, *Mycoplasma pneumoniae* (U00089), *Mycoplasma capricolum* (U51235), *Mycoplasma genitalium* (L43967), are aligned with CLUSTAL program [12]. Three degenerate primers, KF1: 5'-GAYYTWGGWACYACYAAYTC-3' (forward primer); KF2: 5'-GCWGTTATTACHGTWCCTGC-3' (forward primer); KR1: 5'-GWTAAWGGRGTWACRTCYAA-3' (reverse primer), were designed based on the sequences of ATPase domain of *dnaK* gene to amplify PnWB phytoplasma *dnaK* gene fragment with semi-nested PCR method. 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). The first round of PCR was subjected to 35 cycles at the following parameters: denaturation for 30s at 94℃, annealing for 60s

at 42℃ and extension for 30s at 72℃, the total DNA from healthy or PnWB phytoplasma-affected periwinkle plant tissues as template, and KF1 and KR1 as primers in GeneAmp PCR System 2400 (Perkin Elmer, CA). The PCR product of the first round was purified with QIAquick PCR purification kit (Qiagen GmbH, Germany). In the second round PCR of 25 cycles, the purified PCR product was used as template, KF2 and KR1 as primers, and the annealing temperature was raised to 50℃. The amplified PCR product was predicted to have a size around 750 bp. The PCR product was purified, cloned directly into a TA cloning vector (pCR II) (Invitrogen Corporation, CA) and then sequenced.

Genomic library screening

The amplified 750 bp *dnaK* 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 a 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 recombinant phages (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. The filters were hybridized with DIG-labeled probe following the manufacturer's instructions. Plaques giving positive signals were isolated and subjected to secondary and tertiary screenings to ensure plaque purity.

The recombinant pBluescript SK(-) phagemid containing *dnaK* gene were selected and excised from the lambda Zap II vector after an *in vivo* excision using the EsAssist helper phage system according to the manufacturer's instructions (Stratagene).

Southern hybridization

For Southern hybridization, total DNA from healthy periwinkle and PnWB phytoplasma affected periwinkle plants were digested with restriction enzymes *Bam*HI, *Eco*RI or *Xba*I (Boehringer Mannheim GmbH). Three micrograms of the digested DNA were electrophoresed in a 0.8% agarose gel. After denaturation and neutralization of the agarose gel, the DNA fragments were blotted onto a nylon membrane (Micron Separation Inc.) by capillary transfer. The probes used in genomic library screening were also applied in Southern hybridization. Hybridization was performed under low and high stringency at 55℃ and 68℃, respectively[5, 20].

DNA sequence analysis

Nucleotide sequencing was performed with the BigDye Terminator Cycle Sequencing Kit (Perkin Elmer) using ABI PRISM 310 Genetic Analyzer (Perkin Elmer). The entire nucleotide sequence of the PCR-amplified fragments and the selected recombinants were read on both strands. Homologous sequences were searched in GenBank database using program BLAST. Furthermore, the nucleotide sequence was analyzed with the computer program Lasergene (DNASTAR Inc., WI) for ORF searching, codon usage analysis and secondary structure prediction. The deduced amino acid sequences of putative ORFs were compared with those of the related organisms available in the GenBank.

RESULTS AND DISCUSSION

Amplification of a specific *dnaK* gene fragment of PnWB phytoplasma

After the first round of PCR, multiple DNA fragments were obtained. Thus, the second pair of primers were used for amplifying a phytoplsma specific gene fragment using the semi-nested PCR strategy. After a second round of PCR, a specific PCR product of about 750 bp in size was amplified using the purified and diluted first round PCR product as a template. The 750 bp PCR product was then sequenced and aligned with other prokaryotic *dnaK* genes. The PCR fragment contains nucleotide sequences of the highly conserved region and ATPase domain of the *dnaK* gene, and shares more than 80% nucleotide sequences identity with those of other prokaryotic dnaK genes. The results indicate that the PCR fragment should be the *dnaK* gene fragment of PnWB phytoplasma. An *Eco*RI restriction site was identified in the 750 bp fragment around the 200th nucleotide.

Cloning and nucleotide sequence analysis

In the beginning, none of the selected recombinant phagemid contained the full-length *dnaK* gene. An internal *Eco*RI restriction site was identified in the amplified 750 bp *dnaK* gene fragment and the fragment can thus be cut into a 200 bp fragment and a 550 bp fragment with *Eco*RI. For cloning complete sequences of the *dnaK* gene, the 200 bp fragment was then used as a probe for the second and third screens.

After screening recombinant phages of the PnWB phytoplasma genomic library, five positive clones were obtained and confirmed by Southern hybridization. All 5 clones were identified as sibling clones that contained a 2.7 kb insert and the same nucleotide sequences. The cloned sequence contained the 5' end of the *dnaK* gene and overlapped with 550 bp of the amplified 750 bp-PCR fragment. One of the recombinant plasmids was designated as pBK1-1 for further study. For cloning the full length *dnaK* gene, another *Eco*RI-digested fragment (200 bp fragment) of the amplified 750 bp-PCR fragment was used as a probe for the second screening. Six positive clones were obtained and all contained a 2.3 kb insert. The nucleotide sequence of the 2.3 kb insert contained the 3' end of the *dnaK* gene. One of the recombinant plasmids was designated as pBSK3-1 for further study. The 2.7 kb fragment and the 2.3 kb fragment overlapped with 550 bp and 200 bp of the 750 bp-PCR amplified fragment, respectively. The combined 4,974 bp sequence obtained from the sequence of pBK1-1, pBSK3-1 and the 750 bp fragment contained the full length of the *dnaK* gene. The complete nucleotide sequence of the 4,974 bp was determined from both directions three times and then submitted to the GenBank database with an accession number of AF160726. Based on the universal codon usage, four putative ORFs were identified. The ORF1 contains 831 bp (nts 22-852) and encodes a polypeptide of 276 a. a. with a calculated MW of 32.4 KD; the ORF2 contains 795 bp (nts 936-1,730), encodes a polypeptide of 264 a. a. with a calculated MW of 30.5 KD; the ORF3 contains 1,782 bp (nts 1,723-3,504), encodes a polypeptide of 593 a. a. with a calculated MW of 64.9 KD; and the ORF4 contains 1,107 bp (nts 3,700-4,806), encodes a polypeptide of 368 a. a. with a calculated MW of 41.9 KD. All four ORFs started from the ATG initiation codon and stopped at the translation termination codon TAA. Four putative Shine-Dalgano sequences, which were complementary to the 3' end sequence of 16S rRNA of phytoplasmas, were found upstream of the ORFs (nts 17-20, nts 927-931, nts 1,708-1,711, nts 3,687-3,692; accession no. AF160726), but no consensus -10 and -35 sequences were identified.

The base composition of ORF1 is 36.70 mol% of A, 11.07 mol% of C, 12.39 mol% of G, and 39.83 mol% of T, ORF2 is 43.52 mol% of A, 9.94 mol% of C, 13.96 mol% of G, and 32.58 mol% of T, ORF3 is 37.43 mol% of A, 12.85 mol% of C, 18.07 mol% of G, and 31.65 mol% of T, and ORF4 is 38.30 mol% of A, 11.92 mol% of C, 16.62 mol% of G, and 33.15 mol% of T. A low G+C content of the four ORFs (in the order of ORF1 to ORF4, 23.47%, 23.90%, 30.92%, and 28.55%), 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) (73.19%, 61.36%, 54.30%, 56.79%) and 3'-end (3rd base) (83.70%, 89.02%, 87.86%, 90.49%) of codons were observed, which are similar to the features of other phytoplasmas [17, 21, 33]. Except for ORF1, the AAA lysine codon appeared in the other three ORFs as the most frequent codon. In the four ORFs, 15 tryptophan residues are all encoded by UGG. These results agree with that of a previous study on the ribosomal protein genes for *Oenothera* phytoplasma [21]. In the class *Mollicutes*, mycoplasmas and spiroplasmas both utilize UGG and UGA triplets as tryptophan codons [15, 22], but *Acholeplasma laidlawii* utilizes UGG only [22, 37].

Southern hybridization

In Southern hybridization (Fig. 1), total DNA of healthy periwinkle (lane 1) and PnWB phytoplasma-affected periwinkle digested with *Bam*HI (lane 2), *Eco*RI (lane 3) and *Xba*I (lane 4) were hybridized with the DIG-labeled 750 bp *dnaK* gene fragment. No positive signal was observed for the DNA prepared from healthy plants in the Southern hybridization under both low and high stringencies. According to the hybridization patterns, only one fragment of 15.0 kb and 7.0 kb was hybridized in *Bam*HI and *Xba*I digests, respectively. The result suggested that the PnWB phytoplasma has only one copy of the *dnaK* gene. Two hybirdizaion signals of 12.0 kb and 2.7 kb in size were detected in *Eco*RI digested sample. The result was consistent with the sequencing data that there was an internal *Eco*RI site in the *dnaK* gene of the PnWB phytoplasma.

Sequence analysis of deduced proteins

When the deduced amino acid sequences of the four ORFs were compared with sequences available in GenBank, the amino acid sequences of ORF1, ORF2, ORF3 and ORF4 were found to be highly similar to those of the *hrcA*, *grpE*, *dnaK* and *dnaJ* genes of many eubacteria.

ORF1 shares 42% nucleotide sequence identity and 20% deduced amino acid sequence identity with the *hrcA* gene of aster yellows witches' broom (AYWB) phytoplasma, and 42% nucleotide sequence identity and 20% amino acid sequence identity with the *hrcA* gene of onion yellows (OY) phytoplasma. In numerous organisms, CIRCE element was located upstream to the genes of *dnaK* and *groE* operons [42] in the DNA region corresponding to the 5'-untranslated mRNA [27]. However, the CIRCE-like element has not been identified in *E. coli* and some other Gram-negative bacteria [38]. In this study, no CIRCE element was found in the upstream region of the cloned ORF1. Since there were only 21 nucleotides in the upstream region of the cloned ORF1, the CIRCE element maybe located out of the clone range or may be absent in the PnWB phytoplasma. These are questions that remain to be addressed.

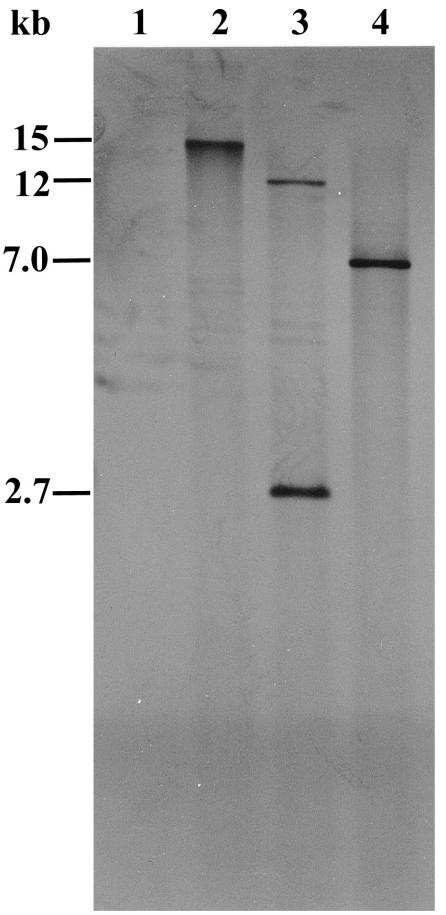

Fig. 1. Southern hybridization of total DNA prepared from healthy periwinkle plant digested with *Eco*RI (lane 1) and diseased periwinkle plant affected with PnWB phytoplasma digested with *Bam*HI (lane 2), *Eco*RI (lane 3), and *Xba*I (lane 4). Sizes (in kb) of the hybridization signals are shown on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

ORF2 shares 55% nucleotide sequence identity and 36% deduced amino acid sequence identity with the *grpE* gene of AYWB phytoplasma, and 55% nucleotide sequence identity and 35% amino acid sequence identity with the *grpE* gene of OY phytoplasma. The secondary structure of the putative PnWB GrpE protein was predicted using the method developed by Garnier et al. [8] (Fig. 2). Most of the *grpE* gene products of various organisms could be divided into three regions [9]. Region 1 is of unknown function. Region 2 is mainly composed of α helix, while region 3, the DnaK binding domain, is mainly composed of β sheet. The putative secondary structure of PnWB phytoplasma GrpE was similar to those of other GrpE proteins.

ORF3 shares 68% nucleotide sequence identity and 63% deduced amino acid sequence identity with the *dnaK* gene of AYWB phytoplasma (GenBank accession no. CP000061), and 66% nucleotide sequence identity and 60% amino acid sequence identity with the *dnaK* gene of OY phytoplasma (GenBank accession no. AP006628). It reveals that the gene organization and the nucleotide sequence in conserved region of ORF3 are similar to those

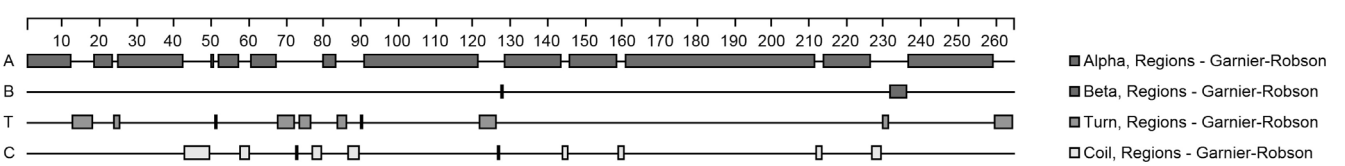

Fig. 2. Secondary structure of the deduced GrpE protein of PnWB phytoplasma predicted by Garnier-Robson method. A, α helix; B, β sheet; T, β turns; C, coiled region.

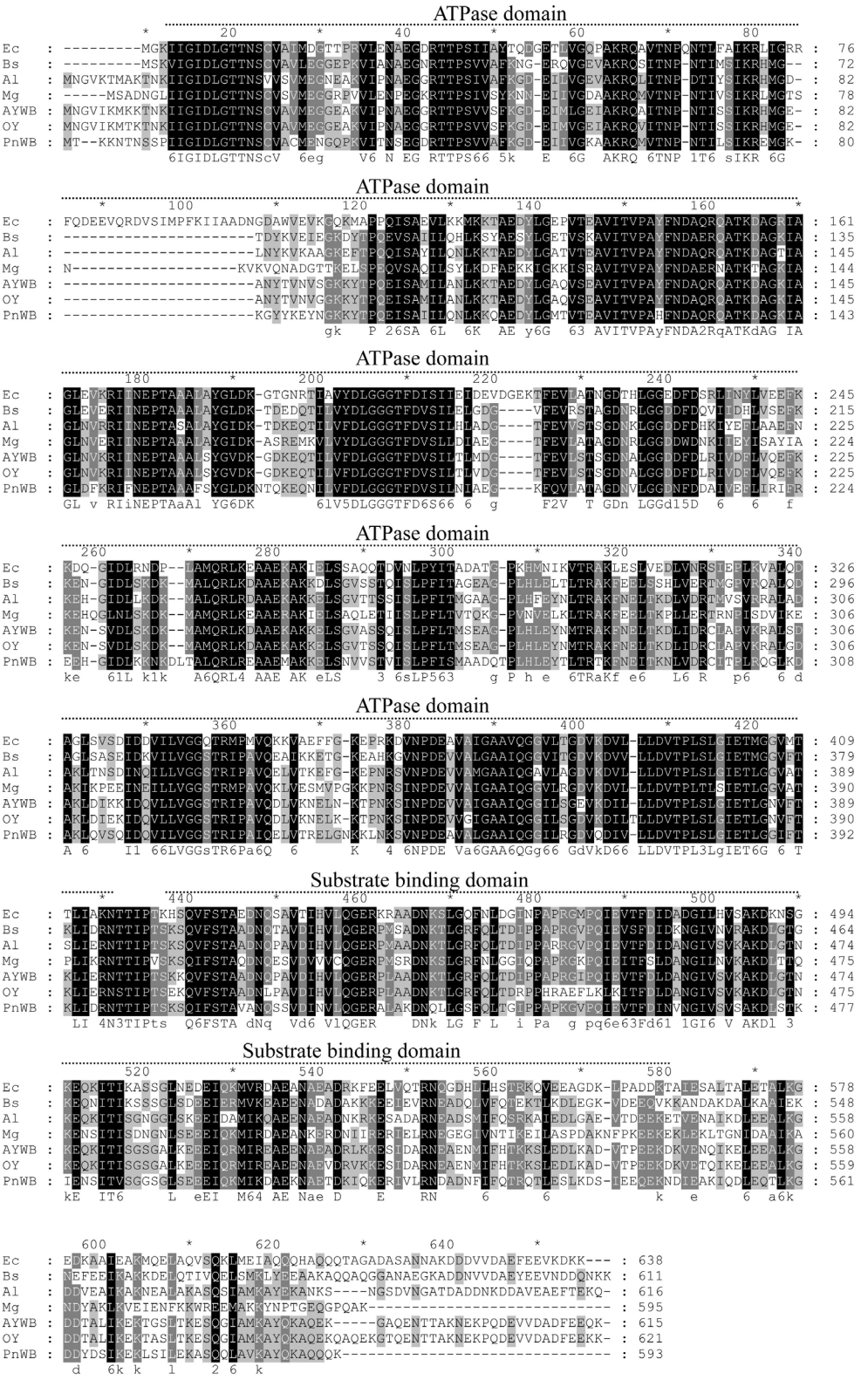

Fig. 3. Multiple amino acid sequence alignment of the putative *dnaK* gene of PnWB phytoplasma (GenBank accession no. AF160726) and *dnaK* gene of *Escherichia coli* (Ec) (AP009048), *Bacillus subtilis* (Bs) (M84964), *Acholeplasma laidlawii* (Al) (AF281816), *Mycoplasma genitalium* (Mg) (L43967), onion yellows phytoplasma (OY) (AP006628), and aster yellows witches' broom phytoplasma (AYWB) (CP000061) conducted by CLUSTAL sequence analysis program. Residues shaded in black are conserved in those of all 7 species compared, residues shaded in dark grey are conserved in those of 6 species, and residues shaded in light grey are conserved in those of less than 6 species. Predicted functional domains are indicated and shown by dotted lines above the sequences.

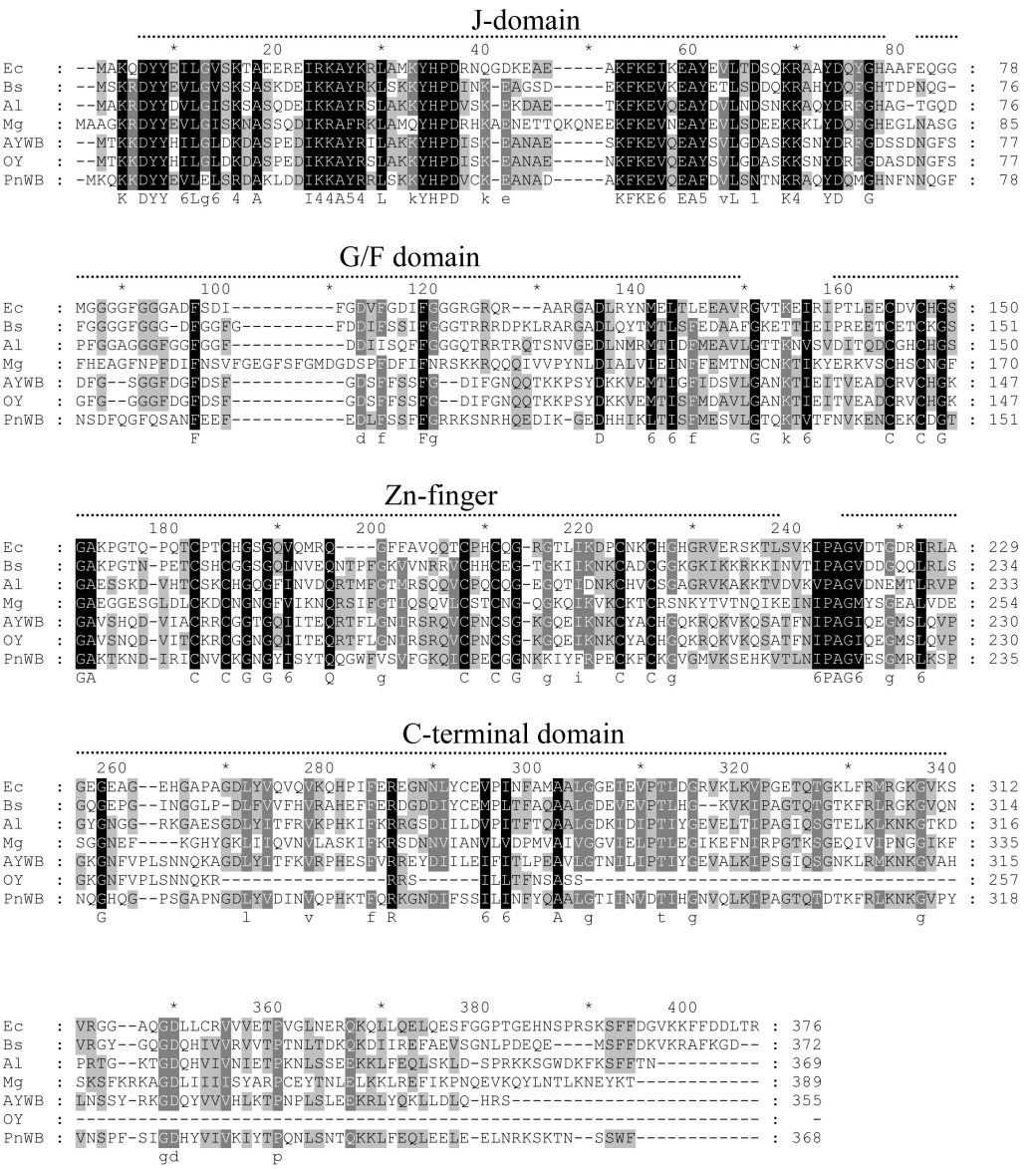

Fig. 4. Multiple amino acid sequence alignment of the putative *dnaJ* gene of PnWB phytoplasma (GenBank accession no. AF160726) and *dnaJ* gene of *Escherichia coli* (Ec) (AP009048), *Bacillus subtilis* (Bs) (M84964), *Acholeplasma laidlawii* (Al) (AF281816), *Mycoplasma genitalium* (Mg) (L43967), onion yellows phytoplasma (OY) (AP006628), and aster yellows witches' broom phytoplasma (AYWB) (CP000061) conducted by CLUSTAL sequence analysis program. Residues shaded in black are conserved in those of all 7 species compared, residues shaded in dark grey are conserved in those of 6 species, and residues shaded in light grey are conserved in those of less than 6 species. Predicted functional domains are indicated and shown by dotted lines above the sequences.

of other *dnaK* genes. The amino acid sequences of ORF3 and ORF4 were aligned with those of the *dnaK* and *dnaJ* genes of *Escherichia coli* (Ec) (GenBank accession no. AP009048), *Bacillus subtilis* (Bs) (M84964), *Acholeplasma laidlawii* (Al) (AF281816), *Mycoplasma genitalium* (Mg) (L43967), OY phytoplasma (AP006628), and AYWB phytoplasma (CP000061), and the results were shown in Figs. 3 and 4. ORF3 contains an ATPase domain and a substrate binding domain (Fig. 3). In addition, PnWB phytoplasma DnaK also has a 23-aa deletion in the N-terminus region that is characteristic of Gram-positive bacteria (Fig. 3).

ORF4 shares 57% nucleotide sequence identity and 41% deduced amino acid sequence identity with the *dnaJ* gene of AYWB phytoplasma (GenBank accession no. CP000061), and 69% nucleotide sequence identity and

30% amino acid sequence identity with the *dnaJ* gene of OY phytoplasma (GenBank accession no. AP006628). ORF4 contains four regions, a J-domain and a G/ F motif interact with DnaK, whereas a Zn finger and the C-terminal domain function as substrate binding regions (Fig. 4). All four regions are functional domain and responsible for most of the biological functions of the DnaK and DnaJ proteins, thus it was suggested that ORF3 and ORF4 may encode the DnaK and DnaJ molecular chaperones of PnWB phytoplasma.

Chromosomal arrangement of *hrcA-grpE-dnaK-dnaJ* genes in PnWB phytoplasma

As mentioned above, the four genes, *hrcA*, *grpE*, *dnaK* and *dnaJ*, were linearly arranged in PnWB chromosome in the order of 5'-*hrcA* (0.83 kb)-*grpE* (0.80 kb)-*dnaK* (1.72 kb)-*dnaJ* (1.11 kb)-3'. The organization of these genes in PnWB phytoplasma is similar to those in several eubacteria including *Bacillus subtilis* [14, 39], *Clostridium acetobutylicum* [28, 31], *Mycoplasma capricolum* [6], AYWB phytoplasma [3] and OY phytoplasma [30]. Notably, the chromosomal arrangement of these four genes was not conserved in all Mollicutes. In *Mycoplasma genitalium* [7, 26] and *Mycoplasma pneumoniae* [13], those genes were located in separate transcription unit. In Gram-positive bacteria, *hrcA*, *grpE*, *dnaK*, and *dnaJ* of *B. subtilis* [14, 39] and *orfA*, *grpE*, *dnaK*, *dnaJ*, *orfB*, *orfC*, *orfD* of *C. acetobutylicum* [28, 31] were located in the same operon, the *dnaK* operon; however, there were no characteristic promoter sequences identified in the cloned 4,974 bp sequence of PnWB phytoplasma. In *B. subtilis* and *C. acetobutylicum*, a transcription terminator is present immediately downstream of the *dnaK* gene [28, 39]. An inverted repeat sequence found downstream of the *dnaK* gene (nts 3,622-3,635, nts 3,638-3,651, accession no. AF160726) of PnWB phytoplasma was predicted to be a terminator, suggesting *hrcA*, *grpE*, *dnaK*, and *dnaJ* might organize into a large transcriptional unit in PnWB chromosome, and the 5' end of the operon was not cloned in this study.

Heat shock proteins protect cells from damage caused by the accumulation of misfolded and unfolded proteins under elevated temperatures. DnaK/ DnaJ/ GrpE and GroES/GroEL are the two major cytoplasmic chaperone systems that facilitate the proper folding of newly synthesized proteins. Here we have shown that the *dnaK* operon exists in PnWB phytoplasma and contains four genes arranged in the order of *hrcA-grpE-dnaK-dnaJ*. The function and regulation mechanism of the *dnaK* operon still need to be studied further.

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摘 要

朱佩文[1]、陳紹寬[1]、陳武揚[1]、林長平[1, 2]. 2007. 花生簇葉病菌質體 molecular chaperones GrpE, DnaK 和 DnaJ 基因之選殖與分析. 植病會刊 16: 215-224. ([1]台北市 國立台灣大學植物病理與微生物學系；[2]聯絡作者，電子郵件：cplin@ntu.edu.tw；傳眞：+886-2-23661980)

本實驗從事花生簇葉病菌質體 *dnaK* 和 *dnaJ* 基因之選殖研究。實驗中根據八種細菌之 *dnaK* 基因高保守性區域設計出 PCR 引子 KF1, KF2, KR1，以花生簇葉病菌質體 DNA 爲模板，先以 KF1/ KR1 爲引子對進行 PCR 反應，再繼續以 KF2/ KR1 爲引子對其 PCR 產物進行半巢式 (semi-nested) PCR，如此可增幅出一大小爲 750 bp 之 PCR 產物，並以其全長或將其經 *Eco*RI 酵解後之 200 bp片段作爲核酸探針，針對以內限制酶 *Eco*RI 構築的花生簇葉病菌質體基因庫進行篩選，分別得到含 2.7 kb 嵌入片段之選殖株重組質體pBK1-1 及含有 2.3 kb 嵌入片段之選殖株重組質體 pBSK3-1。對上述之 750 bp 片段、pBK1-1 及 pBSK3-1之嵌入片段進行核苷酸序列分析，可發現共有四個完整的 ORFs (open reading frames)。將各 ORFs 核苷酸序列推衍爲胺基酸序列，分別和 aster yellows witches' broom phytoplasma (AYWB phytoplasma) 等物種之序列進行比對，發現各 ORFs 分別與 *hrcA*, *grpE*, *dnaK* 及 *dnaJ* 等基因之序列最爲相似，且在序列分析中均可找到相同之基因結構與主要之功能區域 (domains)。基於此等基因之排列與序列特性，本研究指出 molecular chaperon Hsp70 應存在於花生簇葉病菌質體中。

關鍵詞：Mollicutes 綱、*dnaK* 操縱子、半巢式聚合酵素連鎖反應