

## FULL LENGTH RESEARCH ARTICLE

**Isolation and characterization of the ATP-binding cassette (ABC) transporter system genes from loofah witches' broom phytoplasma**CHUN-LIN HUANG<sup>†</sup> & KUO-CHIEH HO*Department of Life Science, Institute of Plant Biology, National Taiwan University, Taipei, Taiwan**(Received 26 July 2006)***Abstract**

A clone containing a 3903 bp *Eco*RI-restriction fragment was obtained from a  $\lambda_{ZAP}$  genomic library of loofah witches' broom (LfWB) phytoplasma by plaque hybridization using a PCR fragment as a probe. Sequence analysis revealed that this fragment contained three open reading frames (ORFs). The deduced amino acid sequences of ORF 1 and ORF 2 showed a high homology with the ATP-binding proteins of the ABC transporter system genes of prokaryotes and eukaryotes, and encoded proteins with a molecular mass of 36 and 30 kDa, respectively. Based on amino acid sequence similarity, secondary structure, hydrophilicity and a signal peptide sequence at the N-terminus, we predicted that ORF 3 might encode a specific solute-binding prolipoprotein of the ABC transporter system with a molecular mass of 62 kDa. The cleavage site of this prolipoprotein signal peptide was similar to those of gram-positive bacteria. In addition to nutrient uptake, ABC transporter systems of bacteria also play a role in signal transduction, drug-resistance and perhaps virulence. The possible implications of the system to the survival and the pathogenesis of phytoplasma were discussed.

**Keywords:** *Loofah witches' broom (LfWB), phytoplasma, ATP-binding cassette (ABC) transporter system, open reading frames*

**Database accession number:** *AF086618 (P36), AF086619 (P30), AF086620 (P62)*

**Introduction**

Phytoplasmas, a member of mollicutes, are the causal agents of more than 200 diseases of higher plants (Kirkpatrick 1989; McCoy et al. 1989). They have remained uncultured *in vitro* and their inability to grow in culture medium has severely hindered their studies (Lee and Davis 1986). As a result, phytoplasmas are the most poorly characterized groups of plant pathogens. Although phytoplasmas' host ranges (plant and insect) and symptomatology (phenotype differences) are similar, their genotype and genome sizes (600–1200 kDa) are diverse (Razin 1992).

Phytoplasmas were known previously as mycoplasma-like organisms (MLOs). However, phylogenetic relationships based on the sequences of the full-length 16S rRNA, ribosomal protein genes and the 16/23S spacer regions revealed that phytoplasmas form a large discrete monophyletic clade, paraphyletic to the

*Acholeplasma* species, within the *Anaeroplasmata* clade rather than *Mycoplasma* (Tully 1993; Gundersen et al. 1994; Sear and Kirpatrick 1994; Ho et al. 2001). Recently, phytoplasma can be detected, identified and classified clearly by molecular methods. While these phylogenetic advances continue, research has focus on the pathogenicity genes. These result in the novel approaches to achieve effective control of phytoplasma diseases (Kirkpatrick and Smart 1995; Lee et al. 2000; Wagner et al. 2001) and the completion of two phytoplasma genome sequences (Oshima et al. 2004; Bai et al. 2006).

The ATP-binding cassette (ABC) superfamily is one of the largest protein families found in living systems, with several hundred different genes identified to date in organisms ranging from bacteria to man. Typically, ABC transporters utilize the energy of ATP to pump substrates across the membrane against

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a concentration gradient. The common protein components of the ABC-type uptake systems include one or two transmembrane proteins which usually span the membrane five to six times each, one or two peripheral membrane ATP-binding proteins in the cytoplasmic side and a high-affinity extra-membrane solute-binding protein (Higgins 1992; Locher 2004; Khwaja et al. 2005). In gram-negative bacteria, the ligand-specific binding protein is soluble and periplasmic. In gram-positive bacteria, the soluble-binding protein is also extracellular and anchors to the membrane via an N-terminal hydrophobic lipid extension. The transmembrane protein components are believed to form solute-specific channels; the peripheral membrane ATP-binding proteins energize the systems and sometimes serve regulatory roles, and the ligand-binding proteins confer specificity and high affinity for the substrates to the transporter systems (Ames 1986; Shuman 1987; Tam and Saier 1993; Monnet 2003). In eukaryotic cells, ABC transporters have been predominantly found in the plasma membrane where they catalyze the efflux of various compounds out of the cell. Other ABC proteins are located in intracellular organelles such as peroxisomes, mitochondria, the endoplasmic reticulum and vacuoles; some of these proteins mediate the compartmentation of compounds into the organelles (Davies and Coleman 2000; Young et al. 2001).

The ABC transporters can transport a remarkable variety of substrates, including ions, carbohydrates, lipids, antibiotics, anti-cancer drugs, pigment molecules and even large peptides. They participate in many biological functions, e.g. phosphate regulation in *Escherichia coli* (Saier 1993), chemical signaling between *Agrobacterium tumefaciens* and its plant host (Ankenbauer and Nester 1990; Cangelosi et al. 1990), control of *Bacillus subtilis* sporulation (Perego et al. 1991; Koide and Hoch 1994), transport of sex pheromones in yeasts (McGrath and Varshavsky 1989), and chloride via the cystic fibrosis transport regulator protein in mammals (Riordan et al. 1989; Tata et al. 1991). The vast majority of studies on ABC transporters have been driven by their diverse importance.

In this communication, we reported the cloning and characterization of a DNA fragment containing the ABC transporter system genes from loofah witches' broom (LfWB) phytoplasma. The possible implications of the system to the survival and the pathogenesis of phytoplasma were also discussed.

## Materials and methods

### *Bacterium and plant*

The LfWB phytoplasmas were maintained by graft inoculation in periwinkles (Chen and Ho 1997). The original diseased plant was provided by Dr H.-J.

Su, Professor of Department of Plant Pathology and Microbiology, National Taiwan University, Taiwan.

### *Phytoplasma genomic library construction and screening*

The following methods, including healthy or diseased plant DNA extraction, phytoplasma DNA purification and phytoplasma genomic library construction were done as previously described (Ho et al. 2001).

The library was screened by plaque hybridization using a <sup>32</sup>P-labelled PCR fragment amplified on the LfWB phytoplasma DNA with primers RN1 (5'-CGCTTAGAGTTTAGGTGA-3') and RN2 (5'-CCAACCAAAGCTTC-3'), synthesized according to the DNA sequence of ribonuclease III of *Mycoplasma genitalium* (Fraser et al. 1995). The positive plaque areas were selected and rescreened until a single, isolated plaque could be picked up.

### *Southern blot analysis*

Phytoplasma DNA (3 µg) or plant DNA (10 µg) were digested with different restriction enzymes and subjected to Southern blot analysis performed at 42°C in the presence of 50% formamide and 0.1% SDS using the <sup>32</sup>P-labelled DNA probe. The filter was washed with 0.1 × SSC (1 × SSC: 150mM NaCl, 15 mM sodium citrate) containing 0.1% SDS at 50°C. The hybridized bands were detected by exposing the filter to a PhosphoImager screen (PhosphoImager 425; Molecular Dynamics).

### *Sequence determination and analysis*

For DNA sequencing, the recombinant phage was converted into a phagemid by *in vivo* excision according to manufacturer's instructions (Stratagene). The sequence of DNA insert in the recombinant phagemid was determined by a DNA automated sequencer (ABI Prism Model 377, v.30; Applied Biosystems) using a step-by-step procedure in which synthetic primers for forward sequencing were designed from previously released sequences. DNA sequence analysis was performed using DNASTar software (DNASTAR). The deduced amino acid sequences were analyzed through the BLAST of National Center for Biotechnology Information server (<http://www.ncbi.nlm.nih.gov/BLAST>), and submitted to the 3D-PSSM fold recognition server (<http://www.sbg.bio.ic.ac.uk/~3dpssm/>) for prediction of secondary structure if it is necessary (Kelley et al. 2000). The alignment of multiple sequences was performed by the program Clustalx ver 1.8 (Thompson et al. 1997).

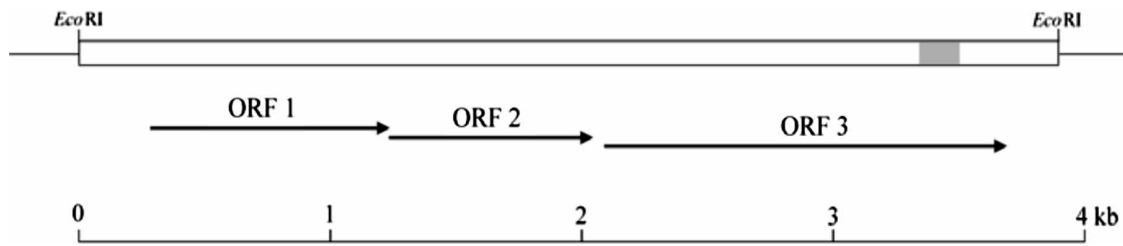


Figure 1. Schematic representation of the 3903 bp cloned-DNA fragment. Arrows indicate the lengths and orientations of the three ORFs. The gray area is the PCR fragment RN-P used as a probe for the hybridization analyses.

## Results and discussion

### *Nucleotide sequence analysis of the cloned DNA fragment*

A 170-bp PCR product, named RN-P was amplified on the phytoplasma DNA. Three clones were obtained using RN-P as a probe to screen an *EcoR*I-genomic library of LfWB phytoplasma constructed on  $\lambda$ ZAP vector. The DNA sequences indicated that these clones contained the same DNA insert of 3903 bp.

The nucleotide sequence of the DNA insert was composed of 76.53% A + T which corresponded with the low G + C content of phytoplasma genome. No strong homology was found by comparing the nucleotide sequence with those deposited in GenBank. However, the deduced amino acid sequence revealed that this 3903-bp DNA insert contained three open reading frames (ORFs) (Figure 1). The ORF 1, from nucleotide 290 to 1237, encoded a protein of 36 kDa (designed as protein P36). The ORF 2, from nucleotide 1237 to 2031, had one base overlapping with ORF 1 and encoded a protein of 30 kDa (designed as protein P30). The ORF 3, from nucleotide 2090 to 3688 and separated by 60 nucleotides from ORF 2, encoded a protein of 62 kDa (designed as protein P62). All three ORFs had an ATG as a start codon, which was preceded by a ribosome binding site. There was a putative -10 sequence and a putative -35 sequence upstream from the ORF 1, however, no obvious sequence for a rho-dependent or -independent termination site was found downstream from the ORF 3 (Figure 2). Transcription termination at indiscrete sites has been reported for several protein-encoding genes and ribosomal genes of archaeobacteria, where transcription stops within or near a T-rich sequence (Ho et al. 2001 and references therein). It was possible that the transcription of this ABC transporter operon terminated in one of several pyrimidine-rich regions downstream from the ORF 3, as found in ribosomal gene of the same organism (Ho et al. 2001).

The primers RN1 and RN2 were originally designed from mycoplasma sequence to screen the library for the gene encoding ribonuclease III of LfWB phytoplasma. Instead, the DNA insert containing

ABC transporter genes was obtained due to the existence of partial sequence homologies.

### *P36 and P30 have homology with ATP-binding proteins*

The deduced amino acid sequences were used to search for protein homologies by BLAST from protein sequence database. Proteins P36 and P30 displayed a significant homology with several ATP-binding proteins. The features of ATP-binding proteins including (1) Walker A motif, a glycine-rich loop involved in ATP binding; (2) the ABC signature motif or C motif; (3) the Walker B motif that is associated with many nucleotide-binding proteins; and (4) a switch region characterized by an invariant histidine residue or H motif, which was suggested to be involved in ATP hydrolysis (Higgins 1992; Zhao et al. 2004) were found in P36 and P30. P30 has shorter sequence length truncating at C-terminus, as it's homolog, DppD of onion yellow (OY) and aster yellows witches'-broom (AYWB) phytoplasmas (Figure 3). Many truncated proteins have been found in the OY and AYWB phytoplasma genomes (Bai et al. 2006). This might be the unique feature of phytoplasma associated with their small genome size.

Hydropathic analysis by the method of Kyte and Doolittle (1982) revealed that P36 and P30 were fairly hydrophilic peripheral membrane proteins as the ATP binding proteins of complex bacterial transporter system (Figure 4A, B). P36 and P30 could form a common engine, which binds and hydrolyzes ATP, and energizes unidirectional substrate transport as the common architecture of ABC transporters. This engine is attached to a specialized translocon composed of two transmembrane proteins.

### *P62 is a hypothetical extracellular solute-binding lipoprotein*

The BLAST database search result showed that the P62 was most similar to the solute-binding protein DppA of the OY and AYWB phytoplasma (Bai et al. 2006), but with only 54% similarity and 48% similarity, respectively. The DppA of ABC dipeptide transporter system belongs to the extracellular solute-binding lipoprotein cluster 5. The proteins of cluster 5

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gaattcaaatcttttcccaaaataggttatnttaagttaaatattttatgggattta 60
tttagcaattttatntttgattgaataattaaaagcagttctgttaattgctttatntt 120
-35
ttttaaaaaacatttgactattacaaaaaaatggttataatnttttaaacagttaat 180
-10
aaatatttaatactgnttttaataaattntttgcttttagttgtgttttcgactgagata 240
SD P36
ttatnttaaatcatgattacataaaaaatataagaaagtgaataatcgatggtgaaaga 300
M L K D
tcggaatgaaaaatcaaatnttaatcagtgcaagaatttatctaaatnttttttag 360
R N E K Y Q I L I E C K N L S K F F F R
aaagaaattttgcaaatctagtatnttttaaaagctaatgatgatattagttatc 420
K K L F A K S S I F L K A N D D I S L S
tattcataaaaggcaaacnttagccgtagtagtggttcaggaaaatctactnttaggaca 480
I H K G Q T L A V V G G S G K S T L G Q
aactntggtgcaattgaaaaatctacttctggaagaattntttatataaaagacacga 540
T L L Q L E K S T S G K V I Y Y K E Q Q
atctattaatntaaagcgaactatctactaaagatttacaatnttttccaaaatcctta 600
S I N L S E L S T K D L Q I I F Q N P Y
tctatctntgaatcctaaaatnttaatatcagatatcattggcgaaggctnttaataca 660
L S L N P K I L I S D I I G E G L L I H
taatttagtaaaaagtaaaaatgatcctctttataaaacaaaaatnttagatatcatgaa 720
N L V K S K N D P L Y K Q K I L D I M K
aaaaatgcggcgtaaaaaggagttatataatcgttctctgctcaattatcggtggcga 780
K C G V K E E L Y N R S L A Q L S G G E
aagacaaagaatcgtatntgcaagaactnttaattatagaacctaaatntttgnttgta 840
R Q R I A I A R T L I I E P K F I V C D
tgaatntgnttctctnttagatgacagatacaaaaaaaaatnttggaattatgaaaca 900
E I V S L L D V T I Q K Q I L E L L K Q
atntaaagaaaaatntgaattaaactnttntgnttnttactcatgnttagggntgctaa 960
L K K N Y E L T L L F I T H D L G V A K
atntntaagdcgatcaaatntgcaataatgntnttaggtaaatnttagaattaggcccaac 1020
Y L S D Q I A I M Y L G K L V E L G P T
agaaaaaattnttcaaaaacctcaacatcctntatacaatntgaatntgntaaatnttatccc 1080
E K I F T N P Q H P Y T I E L L N S I P
taaatntgattgaatntgntatntcaagcaaaaaatntacattgctaaatntgaaaatntctc 1140
K L I E Y G I S S K K Y I A K Y E N S S
ntatgntntnttatacaataagaaaaaaagagataagntntggcatcaagntntctccaaa 1200
Y D F L Y N K K K E D K D W H Q V S P N
SD P30
tcactntatntttagtactaaaaaaagagcattntaatagaaaatntntagaagtaataat 1260
H F I L C T K K R G Y end
M K I L E V N N
ttacatactntatntgaaactgaaaaagntnttagnttaagctgtacaagntnttctntt 1320
L H T Y F E T E K G L V K A V Q G I S F
tntttagaacaaggaaaaactntggntnttagtccgagaatntggaagntgntaaaagntcaa 1380
Y L E Q G K T L G L V G E S G S G K S Q
acagctaatntctnttntgaaatnttntgaaaaaacacaaaaatntcatcaagggntaaat 1440
T A N S I L K L F E K T Q K I H Q G E I
atntntaataatcaagntnttntctgntnttaatagaaaaaaatntgcaaaagntntcgtgnt 1500
I F N N Q V I S D F N E K K M Q K I R G
aaagagatatccatgntnttcaagatcctnttactagntntaaacctatgntntaaata 1560
K E I S M I F Q D P I T S L N P M F K I
aaagatcaaaatntgaaatntnttnttntcatcaagaatntcactntataagaagntntat 1620
K D Q I M E I L F I H Q E I T Y K E A Y
gaaaaagntntaaatntgntaaaaaaactaaaaatntcataatntgntaaacaaatntgaat 1680
E K A L N M L K K T K I H N C E Q I M N
gnttacccntntcaatntatctgntcggntntgntgntcaagntntgntcaatntgntnta 1740
C Y P Y Q L S G G M C Q R V M I A I A L
atntntgntcaacctaaatnttaatcgtcagntntgaagntntactnttagntnttagntntcgtnt 1800
I C Q P K L L I V D E A T I A L D V I V
caaaaagaaatntntgaatntgntaaagaaatntgntaaagaaatntcaaacagntntnta 1860
Q K E I L N L I K E M Q K E N Q T A V L
ntntaaagntcatgntntaaagntnttntctgntaaatntcagacgatntntatgntntgntg 1920
F I S H D L S V I S E I S D D I I V M L
aaagntntaaatnttagaaaaagcattntactcaacaaatntntaaagntntcctaaacacct 1980
K G K I I E K A S T Q Q I L K D P K H P
tacactcgcactntnttaataatntnttaaaaaaagntntntatnttagntntaataatntaa 2040
Y T R T L L N N F L K T S I I S end
SD P62

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tttatttgattattataaaaaaatatttaaagaaagataaaaataaaaatgatttttaa 2100
                                     M I L K
gcaaaaaattttttatatttagctattatatttttcaggtttatcgatttggggaat 2160
Q K I I L Y L A I I L F S G L S I W G I
tgtttaagtttaagaataaaagatcatttgatagaaaagatagaatgattattgctat 2220
V K F K E Y K D H L Y R K D R M I I A I
ttcaaatcaccctaaaagtttgatttttgtaattcaaaagataactaatctgtttatc 2280
S N S P K S L D F↓C N S K D T N S V Y T
tgattggactttaggttttttcatagcactttacttaaggcgccagaaaatcctcaaga 2340
D W T L G L F H S T L L K A P E N P Q D
taaactgaaaacttatttagtagaagaatgggatttggacagagagaaccgaaaaataaa 2400
K P E N L L V E E W D L D R E N R K I K
agccaaattaaaagatggtattttgttcataacggaaataaaatgactacagaagatgt 2460
A K L K D G I L F H N G N K M T T E D V
aatttttactataacagagctgttgaaaaagaacataaagattttgttaacaatataaa 2520
I F T Y N R A V E K E H K D F V N N I K
atctgttcaaaaaatagatgaacttaatttgaatccattttaaagatttaccctcatt 2580
S V Q K I D E L K F E I H L K D L P P F
ttataattttttttataaaaatgttcagagtottgaataaagcagctatagacgaaaa 2640
Y N F I F Y K M F R V L N K A A I D E N
tgaatcagaaggattaaaaataggaacaggcttttataaattagtttcggtatcaaaaga 2700
E S E G L K I G T G L Y K L V S V S K D
taaaaaagattataatttcgaaaaatttgaaaattatcatgctaaaaatgattctgaata 2760
K K D Y N F E K F E N Y H A K N D S E Y
tttagattttgacaattaccacctaaaataacattaaaagttgatccaagtaacgacaa 2820
L D F D K L P P K I T L K V D P S N D N
taotcttttaacttagaaaaaagaatcgcgatcttattttaagttttccaggtaagaa 2880
N L L N L E K K N I D L I L S F P G K N
tattaatgataatttaagagataaagaatcaaaaaaaaattaaacataaaagaggatcg 2940
I N D N L R D K E S K K K L N I K E D R
acaaactctaaagtttagttatgtatataaatcaagaaaacacaaaagaggcaattag 3000
Q T S K V S Y M Y I N Q E N T K E A I R
aaaaattaatagttcaagctattgatttcgaaaaataaagaagaactaaaaattccaaa 3060
K L I V Q A I D F E K I K E E L K I P N
taaagttgctaatggcggtttattacctctatttttaaaggatcatgataaaacagcaaa 3120
K V A N G G L L P P I L K G H D K T A N
ttatagaatattataataaagaattggctaaaacagggtttcaatcgttaacaggaga 3180
Y R I Y Y N K E L A K T G V Q S L T G E
agaaaaaaaataaacatttttaacatcagaaaatcctgatcttgcggttcaattaaaagt 3240
E K K I N I L T S E N P D L A L Q L K V
aaaaagatcatttagagggaagcaggttttgaagttaaaattaaccaagtaccttcaatga 3300
K D H L E E A G F E V K I N Q V P F N D
tataacaacaattcattactactaatgattataatattttgttttttaggtgaacaaca 3360
I T N K F I T T N D Y N I L F L G E Q H
tgaattgatatatgggtataaaatttttgaagattatttcatacattcagacaaaagatcc 3420
E L I Y G Y K F F E D Y F I H S D K D P
aaaaaaccaaaattttcacatataaaaagaaggaggatcgggacaaaatttcaaaattaat 3480
K N Q N F S H I K E E D A D K I S K L I
aaaagactctaaggaagcttagatgatgattcatttattcaaaaaataaaagaataga 3540
K D S K E A L D D D S F I Q K I K E I E
acaatatttatacaaaaattatacattataccaactttttatgtaactgattatgtttt 3600
Q Y L Y T K L Y I I P T F Y V T D Y V L
aactagtctagagtagataaggaacattttaaataatacatttagtggtgtaaaccc 3660
T S S R V D K E H L K I N T F S G V N P
tagatcattaagatttctcaaaaataagataatattttataaacTTTTTgtatattg 3720
R S L R F I S K end
ttataataggacataataataatgcgatattatcaaaaaaatTTTTTataattttta 3780
                                     1
attgTTTTTTTattTTTTTaaatagTTTTTTTattTTTaaagaaaacaactgatocggtt 3840
      2 → 1
acgactataatcggatctaaaaatttaacttcagaaaaaatagaattcataagaaaaga 3900
                                     ← 2
ttc
    
```

Figure 2. Nucleotide sequence of the 3903 bp cloned-DNA fragment and deduced amino acid sequences of P36, P30 and P62. The putative -10 and -35 regions of the promoter, and the pyrimidine-rich sequences where the transcription possibly terminates are boldfaced. The Shine-Dalgarno (SD) sequences for each gene and the possible lipoprotein signal peptidase cleavage site are underlined. The vertical arrow indicates the processing site of the P62 precursor protein. The inverted sequences of the 3'-untranslated region are numbered and indicated by arrows.

	1		40		80					
DppD_Tpe	-----MTEP-	-LLRVENLKT	YFYTEDG---	---VVKAVDG	VSFEVREGET	LGIVGESGSG	KSVTSLSIMR	LLD-QNGKIV	66	
DppD_Tte	-----MARN-	-IVEFRNLKT	YFYTEEG---	---VVKAVND	VVSFSIREGET	VCVVGESGCG	KSVTALSIMR	LQSPPGKIV	67	
OppD_Bcl	-----MARQN	RLLEVENLKT	YFHTENG---	---TVPSVDG	VSFHVDGRGT	VAIVGESGSG	KSVTFSISMG	LVS-PPGKIE	68	
OppF_Bsu	-----MNELTE	KLLEIKHLKQ	HFVTPRG---	---TVKAVDD	LSFDIYKGET	LGLVGESGCG	KSTTGRSIIIR	LYE-----AT	65	
OppF_Bli	-----MAE	KLLEIKNLKQ	HFSTPKG---	---IVKAVDG	ISFDIYKGET	LGLVGESGCG	KSTTGRSIIIR	LYQ-----AT	62	
OppD_Gka	-----MARQ	KLLEVKNLQ	YFPAGRGQ---	---LVKAVDG	VTFDIYKGET	FGLVGESGCG	KSTTGRTIIR	LYE-----AT	64	
DppF_AYWB527	-MTSSN---Q	VLIEIKNLSK	NFSIKKNFLK	PDTLLKANQN	INLSIFKGET	LSVVGSGSG	KSTLQGVLLQ	LIK-----PT	71	
P36		MLKDRNEKYQ	ILIECKNLSK	FFFRKKLFAK	SSIFLKANDD	ISLSIHKQGT	LAVVGGSG--	KSTLQGTLLQ	LEK-----ST	73
DppD_AYWB528	-----M	SLLKVINLHT	YFETKKG---	---LIKAVCG	VSFEVQKGT	LGIVGESGSG	KSQTASISLK	LFE-KNQKIY	64	
DppD_OY192	-----M	SLLKVSNLHT	YFETQKG---	---LIKAVRG	VSFEVQKGT	LGIVGESGSG	KSQTASISLK	LFE-KNQKIY	64	
P30	-----M	KILEVNNLHT	YFETEKG---	---LVKAVQG	ISFYLEQKGT	LGLVGESGSG	KSQTANSILK	LFE-KTQKIH	64	
					Walker A					
	81		120				160			
DppD_Tpe	DG-KIIFKGR	-----	-----NLEL	SESEMRKIR-	-GKEIAMIFQ	EPMVALNPVF	TIGDQIMEAI	ILHQNVS-EK	127	
DppD_Tte	GG-EIIFDGR	-----	-----DILKL	SDAEMRRIR-	-GNEIGMIFQ	EPMTSLNPVL	TIGDQLMEPL	MLHKHMT-KK	128	
OppD_Bcl	AG-HIRFDGT	-----	-----ELTS	SERKMRKVR-	-GNEIAMIFQ	EPLTSLNPVF	TVGHQISEAI	LLHQDTK-KA	129	
OppF_Bsu	DG-EVLFNGE	-----	-----NVHGRK	SRKKLLEF--	-NRKMQMIFQ	DPYASLNPRM	TVADIIAEGI	LIHKLAKTKK	127	
OppF_Bli	SG-EVLFKGK	-----	-----NVHDKK	SAKDLEF--	-NRKMQMIFQ	DPYASLNPRM	TVADIIAEGI	DIHGLAKTKK	124	
OppD_Gka	EG-EVLFNQV	-----	-----NVHGKK	SKKELKEL--	-NRKMQMIFQ	DPYASLNPRM	TVADIIAEGI	DIHGLAKTKE	126	
DppF_AYWB527	SGNVFYYKET	TKKAKEFKEK	KTKDIEKIDL	TTLSNKKEKF	LRKDLQIIFQ	DPFSSLNTHL	KISDIIGEGL	LIHKMIKIGE	151	
P36	SGKVIYYKEQ	-----	-----QSINL	SELS-----	-TKDLQIIFQ	NPYLSLNPKI	LISDIIGEGL	LIHNLVKSKN	131	
DppD_AYWB528	QG-EITFENR	-----	-----IISQF	SEKEMQKIR-	-GNEIAMIFQ	DAISSLNPFV	KIKNQIIEVL	MLHKKLD-YD	125	
DppD_OY192	QG-EITFEKR	-----	-----IISQF	SEKEMQKIR-	-GNEIAMIFQ	DAISSLNPFV	KIKNQIIEVL	MLHQKLD-YT	125	
P30	QG-EIIFNQ	-----	-----VISDF	NEKMQKIR-	-GKEISMIFQ	DPITSLNPMF	KIKDQIMEIL	FIHQEIT-YK	125	
	161		200				240			
DppD_Tpe	EARKMAIDLL	RKVGPIPEPEK	RVDEYPHQLS	GGMRQRAMIA	MALSCRPSLL	IADEPTTALD	VTIQAQILEL	MKELQKEYGM	207	
DppD_Tte	EAWNKAIELI	KQVGIPIRAEQ	IMTSYPHEL	GGMRQRIMIA	MAISCDPKLL	IADEPTTALD	VTIQAQILD	LRRLKEEKKM	208	
OppD_Bcl	EARKQGIAML	KRVGIPIRAEQ	VYQSYPHALS	GGMRQVMIA	MALSCNPKML	IADEPTTALD	VTIQAQILRL	LKKISKEVD	209	
OppF_Bsu	ERMQRVHELL	ETVGLN--KE	HANRYPHEFS	GGQRQRIGIA	RALAVDPEFI	IADEPISALD	VSIQAQVNL	MKELQKEKGL	205	
OppF_Bli	ERLERVHELL	NLVGLN--KE	HANRYPHEFS	GGQRQRIGIA	RALAVEPEFI	IADEPISALD	VSIQAQVNL	MKDLQKERGL	202	
OppD_Gka	ERMQRVYELL	ETVGLN--RE	HANRYPHEFS	GGQRQRIGIA	RALAVEPEFI	IADEPISALD	VSIQAQVNL	LKRLQREKGL	204	
DppF_AYWB527	DPKYQKMILD	IMKKGIDNY	LYDCYPHQLS	GGQRQRISIA	RALIIKPKFV	VCDEIVSALD	VSIQSQILNL	LNDLKKDYQI	231	
P36	DPLYKQKILD	IMKKGKVEE	LYNRSLAQLS	GGQRQRISIA	RTLIIKPKFI	VCDEIVSLD	VTIQIQILEL	LKQLKKNYEL	211	
DppD_AYWB528	QAYKKTLDIL	EKVQIPNAQR	VMNSYPHQLS	GGMCQRIMIA	MALVCKPKLL	IADEATTALD	VIVQKEILNL	IACLQEQNT	205	
DppD_OY192	QAYKKTLDIL	EKVQIPNAQR	VMNSYPHQLS	GGMCQRIMIA	MALVCKPKLL	IADEATTALD	VIVQKEILNL	IACLQEQNT	205	
P30	EAYEKALNML	KKTKIHNCEQ	IMNCYPYQLS	GGMCQRVMIA	IALICQPKLL	IVDEATTALD	VIVQKEILNL	IKEMQKENQT	205	
				C motif		Walker B				
	241		280				320			
DppD_Tpe	AIIILITHDMG	VVAEMSDKVA	VMYAGKVVEY	GDVKTIFTEP	KHPYTYALLE	SIPRIDVEQE	RLKS-IPGNV	PDPLNFPPGC	286	
DppD_Tte	ALMLITHDLG	IVAEMADYVV	VMYAGKVIEE	APVRELFKNP	KHPYTRGLLK	AKPVIQRQE	RLYT-IPGVV	PNPIDLGDFC	287	
OppD_Bcl	SIILITHDLG	VVAELVDRVI	VMYAGQIVEQ	ADVYTIFKDP	KHPYTQGLLE	STPKIHLEHD	ELKS-IRGVV	PVPTNMPGTC	288	
OppF_Bsu	TYLFIADHLS	MVKYISDRIG	VMYFGKLVLEL	APADELYENP	LHPYTKSLLS	AIPLPDPDYE	RNRVRQKYD-	PSVHLKGDG	284	
OppF_Bli	TYLFIADHLS	MVKYISDRIG	VMYFGKLVLEL	APADELYENP	LHPYTKSLLS	AIPLPDPDYE	RTRVRKTYD-	PSVHLKGDG	281	
OppD_Gka	TYLFIADHLS	MVKYISDRIG	VMYFGKLVLEL	AESEELYRNP	IHPYTKALLS	AIPLPDPETE	RTRKRIVYD-	PAQHGYKDGE	283	
DppF_AYWB527	TELFITHDLG	VARFLSDRIC	VMHLGKVIEI	APSESIFKNP	YHPYTKQIIN	AIPKLKTQNK	VLEEIPITYE	TKKFRFLFQT	311	
P36	TELFITHDLG	VAKYLSQDIA	IMYLGLKLVLEL	GPTKIFTNP	QHPYTKIINN	SIPKLIIEYGI	SSKKYIAKYE	NSSYDFLYNK	291	
DppD_AYWB528	SVLFITHDLG	VVSQVADDII	VMYRGKIVES	AKTSQILKNP	QHTYTKSLLN	NFLKTGLGYH	NF-----	-----	267	
DppD_OY192	AVLFITHDLG	VVSQVADDII	VMYQGQIVES	AKTSQILKNP	KHPYTKSLLN	NFLKTGLGYH	NV-----	-----	267	
P30	AVLFISHDLS	VISEISDII	VMLKGKIIIEK	ASTQQILKDP	KHPYTRTLLN	NFLKTSIIS-	-----	-----	264	
				H motif						
	321		360							
DppD_Tpe	KFHPRCEFFE	KGKCDVEEPE	LEDLDGNHKV	RCFFWQKLDE	MR-HAKSEV-		334			
DppD_Tte	YFSDRCBYTM	D-VCRKKMPP	LVADENGHKV	ACWLYEG---	----EWKK--		327			
OppD_Bcl	RFHPRCPHAM	D-ICKEKEPM	MVDRDGKAQV	RCWLYAS---	----EKEDVG		330			
OppF_Bsu	TMEFREVKPG	HFVMCTE-AE	FKAFS-----	-----	-----		308			
OppF_Bli	QMELEREVKPG	HFVMCTE-EE	FKAYQSK---	-----	-----		307			
OppD_Gka	ELEFREITPG	HFVLCSE-EE	YKRYRAMYA-	-----	-----		311			
DppF_AYWB527	QKKDLWDHQV	APNHFISCTL	KNKATKM---	-----	-----		338			
P36	KKEDKWDHQV	SPNHFILCTK	KRGY-----	-----	-----		315			
DppD_AYWB528	-----	-----	-----	-----	-----		267			
DppD_OY192	-----	-----	-----	-----	-----		267			
P30	-----	-----	-----	-----	-----		264			

Figure 3. Comparison of P36, P30 and ATP-binding proteins of other microorganisms' ABC transporter systems. The sequences of Walker A, C motif, Walker B and H motif are underlined and indicated. DppD\_Tpe = DppD of *Thermotoga petrophila* (ZP\_01652599); DppD\_Tte = DppD of *Thermoanaerobacter tengcongensis* (NP\_624050); OppD\_Bcl = OppD of *Bacillus clausii* (YP\_177152); OppF\_Bsu = OppF of *B. subtilis* (P24137); OppF\_Bli = OppF of *Bacillus licheniformis* (YP\_078436); OppD\_Gka = OppD of *Geobacillus kaustophilus* (YP\_146668); DppF\_AYWB527 = DppF of aster yellows witches'-broom phytoplasma (YP\_456723); DppD\_AYWB528 = DppD of aster yellows witches'-broom phytoplasma (YP\_456724); DppD\_OY192 = DppD of onion yellows phytoplasma (NP\_950444).

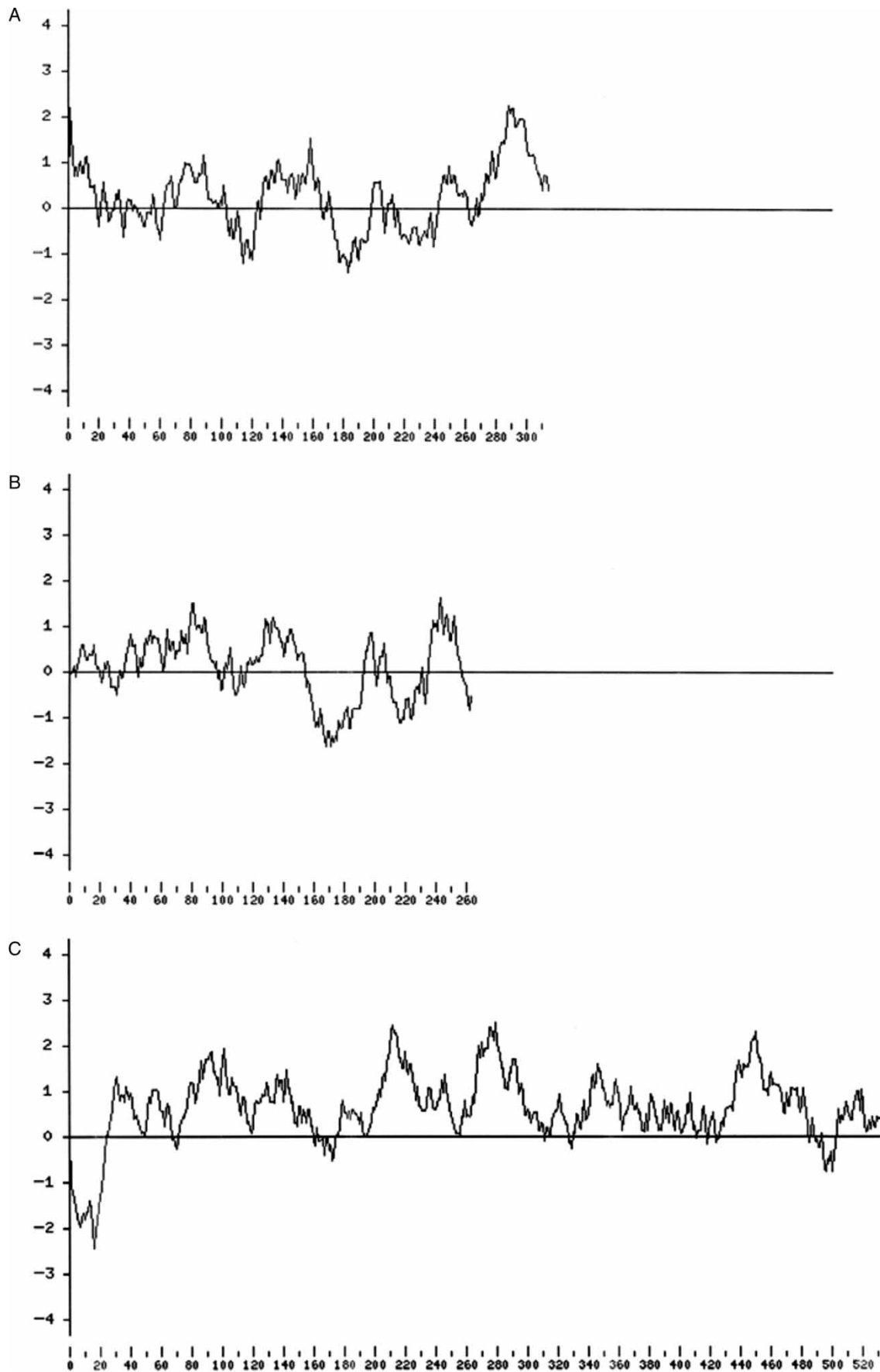


Figure 4. Hydropathy plots of the polypeptides P36 (A), P30 (B) and P62 (C). The hydropathy profiles are obtained according to the method of Kyte and Doolittle (1982), using a window length of 19. Hydrophilic regions are above the line and hydrophobic regions are below.

are peptide- and nickel-binding proteins that the average sequence similarity score is 0.52 and the identity is low (Tam and Saier 1993). The lipoprotein is the key to understanding the function of the ABC transporter system. However lipoproteins seldom show homology between each other. It is hard to determine function from comparison of the protein sequences (Tam and Saier 1993). The extracellular solute-binding receptors of bacteria were grouped into eight clusters based on their sequence similarity, and these groupings were generally found to correlate with the molecular sizes and solute-binding specificities of the proteins. The proteins of cluster 5 are the largest of the solute-binding proteins with a size range of 493–543 residues. They are more than 100 residues larger than any of the other binding proteins (Tam and Saier 1993). The molecular size of P62 was in the range of the cluster 5 binding proteins.

Since moderate amino acid sequence similarity to ABC-type dipeptide transporter protein, P62 was further submitted to the 3D-PSSM fold recognition server for prediction of secondary structure and then aligned to secondary structure elements of proteins with a solved crystal structure. The secondary structure of P62 showed the most similarity to the ABC oligopeptide-binding protein AppA of *B. subtilis* (Levdikov et al. 2005) with an E-value of 1.4e-41 and 100% estimated precision. AppA is a lipid-modified, membrane-anchored extracellular binding-protein that serves as the receptor for the transport system. It plays important roles in the signal pathway leading to the development of competence and sporulation (Levdikov et al. 2005).

The hydropathy analysis revealed that P62 was a hydrophilic protein with an N-terminal hydrophobic signal peptide and had no obvious membrane spanning domains (Figure 4C). Because the phytoplasma has no periplasmic space, there should be some mechanism to anchor hydrophilic solute-binding proteins to the membrane. There are three types of export sequence of membrane proteins: (1) non-cleavable, membrane-spanning sequence; (2) signal peptides with signal peptidase (SPase) I-like cleavage sites; and (3) signal peptides with SPase II-like lipoprotein-cleavage sites. There is no strict consensus sequence in the mycoplasma lipoprotein cleavage site besides a Cys in the +1 position. Leu is common at the -3 position in other eubacteria, but not in mycoplasmas (Cleavinger et al. 1995; Sutcliffe and Russell 1995). The region of the putative cleavage site for signal peptide (Figure 2) in P62 had a sequence L-D-F-C-S-N which was consistent with the consensus sequence (L-Y-Z-cleavage site-C-y-z) of the lipoprotein precursors (Gilson et al. 1988; Sutcliffe and Russell 1995). In these proteins the N-terminal cysteine is modified into a lipo amino acid that is thought to anchor them to the membrane

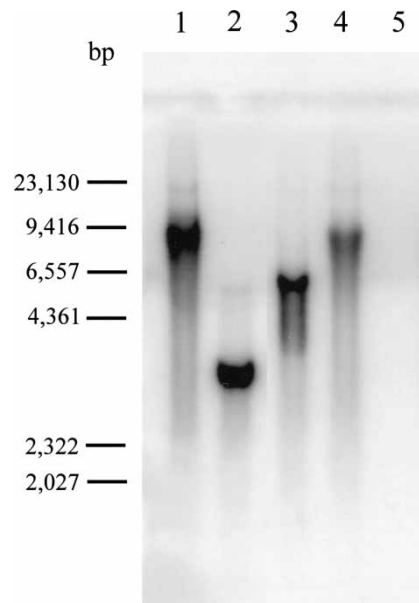


Figure 5. Southern blot analysis of phytoplasma and healthy periwinkle DNA. Lanes 1–4 are the phytoplasma DNA (3 µg/lane) digested with *Bam*HI, *Eco*RI, *Pst*I and *Sal*I, respectively. Lane 5 is the healthy periwinkle DNA (10 µg) digested with *Eco*RI. The probe used was a <sup>32</sup>P-labeled RN-P fragment.

(Nielsen and Lampen 1982). These analyses suggested that P62 was a solute-binding lipoprotein specific for peptides and attached to the membrane via the modified N-terminal as in gram-positive bacteria.

#### Southern blot analysis

In an attempt to find out the copy number of the ABC transporter system genes, phytoplasma genomic DNA digested with each of the following restriction enzyme: *Bam*HI, *Eco*RI, *Pst*I and *Sal*I, and analyzed with a Southern blot probed by a <sup>32</sup>P-labeled RN-P fragment. There was single band in the individual lanes containing phytoplasma DNA but not in the lane of the healthy periwinkle DNA digested with *Eco*RI, suggesting that there was only a single copy of the genes in the phytoplasma genome and the genes were LfWB phytoplasma-, but not host- specific (Figure 5).

Phytoplasmas are obligatory parasites in the phloem of host plants, and have a small genome. The complete genome of *Candidatus* phytoplasma encodes even fewer metabolic functions than that of mycoplasma genomes. The loss of some biosynthetic pathways during the course of evolution results in their inability to be cultured *in vitro*. They must take up their basic nutrients from the environment through transporter systems to survive. The genomes of OY and AYWB phytoplasmas, and *Spiroplasma kunkelii* have many genes encoding transporter systems (Oshima et al. 2004; Zhao et al. 2004; Bai et al. 2006). The ABC transport systems of some bacteria are connected with virulence and pathogenesis in the host (Dudler et al.



1988; Parra-Lopez et al. 1993; Urban et al. 1999; Brown et al. 2001; Liu et al. 2001; Espinasse et al. 2002; Fleissner et al. 2002; Janulczyk et al. 2003). The role of lipoproteins in nutrient transport, cytoadhesion and antigenic variation had been reported in mycoplasmas (Cleavinger et al. 1995; Sutcliffe and Russell 1995). The consumption of metabolites with phytoplasma infection greatly disturbs the metabolic balance of the host cell, and might be a major factor causing disease symptoms (Bai et al. 2006).

In conclusion, we report in this communication an operon isolated from LfWB phytoplasma. The operon contains three structural genes encoding three proteins constructing an ABC transporter which shares a common ABC structure and might have the same mechanism of translocation of specific substrates. Southern blot analysis indicated that this ABC transporter system was LfWB phytoplasma-, but not host- specific. It could be a target for designing therapeutic agents against the pathogen.

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