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Adsorption protein of filamentous bacteriophage ϕXo from *Xanthomonas oryzae*

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Abstract. ϕXo, Xf, ϕLf, ϕXv, and Cf are filamentous bacteriophages isolated in Taiwan. Both ϕXo and Xf specifically infect *Xanthomonas oryzae* pv. oryzae, and ϕLf, ϕXv, and Cf specifically infect *X. campestris* pv. campestris, *X. campestris* pv. vesicatoria, and *X. campestris* pv. citri, respectively. In this study, the ϕXo gene III (*gIII*) encoding the adsorption protein (pIII) was cloned by probing with the ϕLf *gIII*. Sequence analysis revealed that the ϕXo *gIII* is 1,023-nt long and able to encode a pre-protein of 340 aa (35,337 Da), with structural features typical of filamentous phage adsorption proteins: an N-terminal signal sequence (18 aa), a central region (90 aa) containing 38 glycine, 29 aspartic acid and 19 histidine residues, and a C-terminal membrane-anchoring domain (17 aa). The ϕXo pIII purified from the phage particles migrated as a 42-kDa band in SDS-polyacrylamide gel, which is substantially larger than that deduced from the nucleotide sequence, presumably due to the presence of the long stretch of charged residues in the central region. The ϕXo pIII could cross-react with the antiserum specific to ϕLf pIII, which also cross-reacts with ϕXv pIII. Like the situations in ϕLf and ϕXv, the pIIIs of ϕLf and ϕXo are also interchangeable. The *gIII* and the flanking regions of ϕLf, ϕXv and ϕXo are highly homologous and similar in size, but ϕXo has a genome (7.6 kb) larger than that of ϕLf (6.0 kb) and ϕXv (6.4 kb), suggesting that ϕXo is able to accommodate more genes and/or have longer intergenic regions in the remaining part of the genome. Difference in sizes between the pIIIs indicates that ϕXo and Xf, which has a predicted pre-pIII of 488 aa (51,036 Da), are distinct phages.

Keywords: Adsorption protein; Aspartic acid and histidine; Gene III; High content of glycine; Membrane-anchoring domain; Signal sequence; *Xanthomonas.*

Introduction

Several members of *Xanthomonas*, a genus of gram-negative phytopathogenic bacteria, are known to carry filamentous phages, e.g., ϕLf, ϕXv and Cf specifically infecting *X. campestris*, *X. vesicatoria* and *X. citri*, respectively, and Xf and ϕXo both infecting *X. oryzae* (Dai et al., 1980; Kuo et al., 1969; Lin et al., 1994; Tseng et al., 1990). Among them, all isolated in Taiwan, the nucleotide sequence has been determined for the ϕLf and Cf genomes (Kuo et al., 1991; Wen, 1992), whereas the amino acid sequence has been described in an abstract for the Xf gene *III* coding for the adsorption protein (pIII) (Yang and Yang, 1998), although the sequence was not yet available in the database. Like other filamentous phages, they possess a circular single-stranded DNA (ssDNA) genome, produce replicative form (RF) during DNA replication, and propagate without lysis of the host cells (Dai et al., 1980; Kuo et al., 1969; Lin et al., 1994; Model and Russel, 1988; Tseng et al., 1990). Several interesting properties of these *Xanthomonas* phages have also been noticed, including: i) they hold restrictive host specificity, each phage being able to infect only its own host; however, they can propagate in the non-host *Xanthomonas* cells upon electroporation with RF or ssDNA, and the electroporated cells are capable of releasing authentic phage particles (Lin et al., 1994; Yang and Yang, 1997) indicating that host specificity is determined by the early steps of infection, i. e., adsorption and/or penetration, and ii) the genomes of Cf (7.8 kb), Xf (7.4 kb), and ϕXo (7.6 kb) are similar in size, but substantially larger than that of ϕLf (6.0 kb) and ϕXv (6.4 kb) (Kuo et al., 1991; Lin et al., 1994; Wen, 1992; Yang and Yang, 1998). Although Xf and ϕXo infect the same host, comparative study of these two phages has not been performed.

In filamentous phages, such as the best studied Ff phages (the closely related M13, f1 and fd), a phage particle contains about 2,700 copies of the major coat protein (pVIII) and three to five copies each of the four minor coat proteins (pIII, pVI, pVII and pIX), with pIII and pVI located at one end and pVII and pIX located at the other

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(Model and Russel, 1988). pIII (adsorption protein) mediates phage adsorption to pilus (receptor recognition) and is necessary for phage uncoating and DNA penetration into the host cell (Endemann et al., 1992; Endemann and Model, 1995; Stengele et al., 1990), which also requires the function of host proteins TolQ, R and A (Levengood and Webster, 1989; Sun and Webster, 1987; Webster, 1991). In *Xanthomonas* filamentous phages, adsorption and penetration have not been studied in detail. It was only recently that we reported the purification of ϕLf and ϕXv pIIIs and the sequences for the corresponding genes (*gIII*s) and demonstrated that they determine host specificity (Liu et al., 1998; Lin et al., 1999). In addition, interchangeability has been demonstrated between the pIIIs of ϕLf and ϕXv and between the pIIIs of Cf and Xf, which resulted in the change of host specificity (Liu et al., 1998; Lin et al., 1999; Yang and Yang, 1997). In this study, we purified the pIII from ϕXo phage particles and cloned and sequenced the corresponding gene. Western blotting demonstrated that the purified ϕXo pIII could cross-react with the antiserum specific to ϕLf pIII. Sequence analysis revealed that the ϕXo pIII possessed structural features typical of filamentous phage pIIIs. The ϕXo pre-pIII was similar to that of the ϕLf and ϕXv both in size and amino acid sequence, but 148 amino acid residues smaller than that of the Xf pre-pIII.

Materials and Methods

Bacterial Strains, Plasmids and Cultivation Conditions

Xanthomonas campestris pv. campestris strain P20H (Yang et al., 1988) and *X. oryzae* pv. oryzae strain Xo21 (Lin et al., 1994) were the hosts of ϕLf and ϕXo, respectively, and were used separately for phage propagation and as the indicator cells in plaque assay. *Escherichia coli* strain DH5α was used for gene cloning, and strain JM101 was the host for propagating M13 derivatives. LB broth and L agar (Miller, 1972) were the media for growing *Xanthomonas* (28°C) and *E. coli* (37°C). Plasmid pRKG3 (Liu et al., 1998) was constructed previously by cloning the ϕLf *gIII*, within a PCR-amplified 1,147-bp fragment, into the broad-host-range vector pRK415 (Keen et al., 1988), which contained the RK2 origin. To select for plasmids, the media were supplemented with antibiotics, ampicilin (50 μg/ml), kanamycin (50 μg/ml), or tetracycline (15 μg/ml).

Phage and DNA Techniques

Phages were propagated and purified as described by Lin et al. (1999). Double-layer bioassay (Eisenstark, 1967) was performed to determine phage titers. A spot test (Tseng et al., 1990) was carried out to verify phage sensitivity. Restriction endonucleases, T4 ligase and other enzymes were purchased from New England BioLabs and used in accordance with the instructions supplied. Preparation of plasmid and RF DNA, gene cloning, preparation of ^{32}P-labeled probes, Southern hybridization, and transformation of *E. coli* were performed as described by Sambrook et al. (1989). *Xanthomonas* strains were transformed by electroporation (Wang and Tseng, 1992).

Sequence Analysis

Signal sequence and membrane anchoring domain were predicted with the PSORT program (Nakai and Kanehisa, 1991). Hydropathy plot was done by the program of Kyte and Doolittle (1982). Sequence of both strands was determined by the dideoxy chain termination method of Sanger et al. (1977).

Protein Techniques

The ϕXo pIII was purified with a fast protein liquid chromatography (FPLC) system as described for purification of the ϕLf pIII (Liu et al., 1998). Protein was separated by SDS-polyacrylamide gel electrophoresis (Laemmli, 1970). Western blotting was performed as described by Sambrook et al. (1989), using the antiserum prepared by immunizing a rabbit with the FPLC-purified ϕLf pIII (Liu et al., 1998).

Nucleotide Sequence

The nucleotide sequence reported here has been registered in GenBank under accession number AF162859.

Results and Discussion

Cloning and Sequencing of the ϕXo Gene III

To clone the ϕXo *gIII*, we used several restriction endonucleases to cut the ϕXo RF DNA, and the *gIII*-containing fragments were probed with the labeled pRKG3 containing the ϕLf *gIII* (Liu et al., 1998). The smallest fragment showing a hybridization signal was the 1.4-kb *Hin*dIII-*Kpn*I fragment. The position of this fragment was near the middle of the restriction map of the linearized ϕXo RF DNA (Figure 1). The *Hin*dIII-*Kpn*I fragment was subcloned into M13mp18 and mp19, and then the nucleotide sequence on both strands was determined. This fragment contained 1,386 bp with a G+C content of 56.5%, similar to that of the ϕLf genome (Wen, 1992), but deviating from that of the *X. oryzae* chromosome (64.6%) (Bradbury, 1984). The *gIII*-coding region spanned for 1,023 nt, starting with GTG at nt 146 and terminating with TGA at nt 1,168. Eleven nt upstream from the predicted translation initiation codon was a possible ribosome-binding site, 5'-AAGG-3', which was complementary to the 3'-end of the *X. campestris* pv. campestris 16S rRNA (Lin and Tseng, 1997; Figure 2A). This *gIII* was able to encode a polypeptide of 340 aa with a predicted molecular weight of 35,337, a size similar to that of the ϕLf pre-pIII (333 aa, 32,857) and the ϕXv pre-pIII (328 aa, 31,937 Da), but much smaller than that of the Xf pre-pIII (488 aa, 51,036 Da) (Yang and Yang, 1998).

Previously, nucleotide sequence analysis has shown that the upstream flanking regions of ϕLf *gIII* and ϕXv *gIII* are highly homologous, with an identity of 93% (Lin et al.,

1999). With a *Hin*dIII site at their left ends, they are 145 nt in length containing the C-terminal 5 codons (16 nt) of the major coat protein gene (*gVIII*) and the 129-bp *gVIII*/*gIII* intergenic region. Within these intergenic regions are the inverted repeats, resembling a transcription terminator for the upstream cistron (*gVIII*) and the *gIII* promoter (Lin et al., 1999). The *gIII* upstream region of ϕXo *gIII* was also 145-nt long, which shared 93% and 95% identity with the upstream regions of ϕLf and ϕXv *gIIIs*, respectively (Figure 2A). Consequently, sequences identical to the 16-nt C-terminus of *gVIII* and the putative transcriptional terminator present in the corresponding regions of ϕLf and ϕXv were all found in the upstream region of ϕXo *gIII* (Figure 2A). The nucleotide G at 74 nt upstream from the ϕLf *gIII* was identified as the transcriptional start site (Lin et al., 1999). Based on analogy, the same nucleotide G at 74 nt upstream from the ϕXo *gIII* start codon was predicted to be the transcriptional start site (Lin et al., 1999; Figure 2A).

The ϕLf *gIII* is followed by *gVI*, the gene coding for one of the minor coat proteins, after an intergenic region of 42 nt; it initiates with GTG situated 4 nt downstream from a possible ribosome binding site (Liu et al., 1997). In ϕXv, *gIII* is also followed by *gVI*, with a larger intergenic region of 129 nt (Wang, W.-H. and Tseng, Y.-H., unpublished results). In the downstream flanking sequence of ϕXo *gIII* (218 nt), which possessed 86.7% identity to the corresponding region of ϕLf, there was an incomplete open reading frame of 59 codons (Figure 2C). Its deduced amino acid sequence possessed 80.2% identity to that of the ϕLf *gVI*, although it was 40 nt instead of 42 nt behind *gIII* and initiated with ATG instead of GTG. These results indicated that, in *gIII* and the flanking regions, ϕXo had a genome organization identical to that of ϕLf and ϕXv, *gVIII-gIII-gVI* (Lin et al., 1999; Wang, W. -H. and Tseng Y.-H., unpublished results).

The size of the ϕXo RF DNA (7.6 kb) is similar to that of Xf (7.4 kb) and Cf (7.8 kb), but 1.6 kb and 1.2 kb larger than that of ϕLf and ϕXv, respectively (Kuo et al., 1991; Lin et al., 1994; Wen, 1992; Yang and Yang, 1998). However, the *gIII*s and the flanking regions of the ϕLf, ϕXv and ϕXo are similar in length and contain the analogous genes, *gVIII-gIII-gVI*. Therefore, it seems safe to predict that ϕXo may accommodate more genes than ϕLf and ϕXo do or have larger intergenic regions in the remaining part of the ϕXo genome. In contrast, differences were noticed between the ϕXo *gIII* and the *gIII*s of Cf and Xf: i) no homology was found between the ϕXo *gIII* and any open reading frame from the complete sequence of the Cf genome (Kuo et al., 1991), and ii) the ϕXo *gIII* was 444 nt less than the Xf *gIII* (Yang and Yang, 1998). These differences suggest ϕXo to be phylogenetically closer to ϕLf and ϕXv than to Cf, and that ϕXo and Xf, although infecting the same host, are distinct phages.

Amino Acid Sequence Analysis of the ϕXo pIII

The deduced amino acid sequence of the ϕXo pre-pIII shared 52% and 60% identities with that of the ϕLf pre-

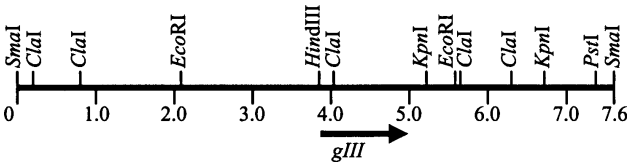

Figure 1. Restriction map of the ϕXo RF DNA and the position of gene III. The 7.6-kb, double-stranded, circular DNA molecule is linearized at the unique *Sma*I site. The position of gene III, within the 1,386-bp *Hin*dIII-*Kpn*I fragment, is indicated by an arrow.

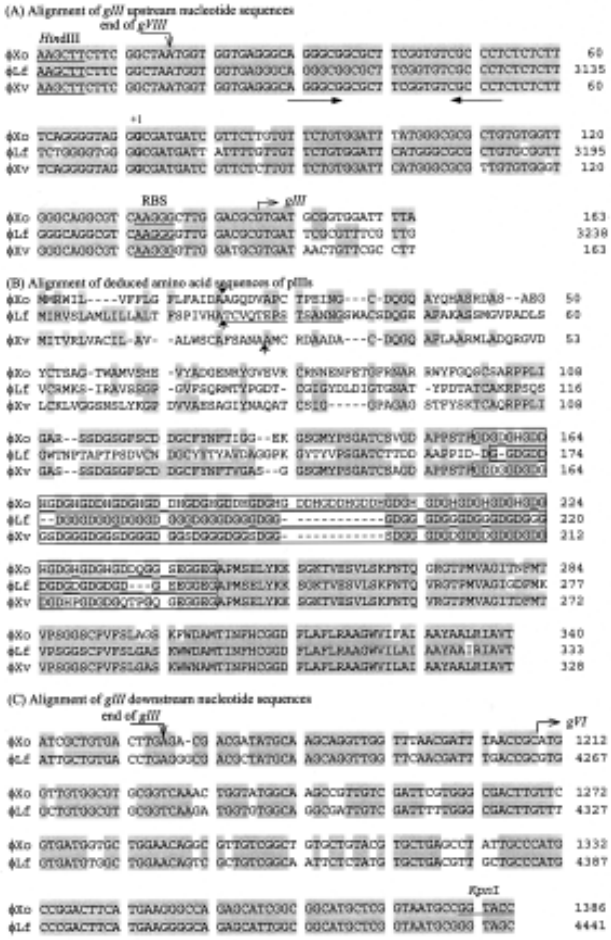

Figure 2. (A) Alignment of the ϕXo *gIII* upstream region with that of the ϕLf and ϕXv. Shown are the left-most region of 163 nt from the sequenced *Hin*dIII-*Kpn*I fragment, including the C-terminus of *gVIII* (16 nt), the intergenic region (145 nt), and the N-terminus of *gIII* (18 nt). Identical nucleotides are shadowed. The inverted arrows indicate the sequences having the potential to form a stem-loop structure resembling the transcription terminator. +1 is the transcription start site G determined for ϕLf *gIII* (Lin et al., 1999). RBS stands for the predicted ribosome-binding site, the Shine-Dalgarno sequence. (B) Alignment of the deduced amino acid sequences of the ϕXo, ϕLf and ϕXv pIIIs. Spaces are introduced for optimal alignments. Identical amino acid residues are shadowed. Vertical arrows indicate the computer-predicted sites for signal peptidase cleavage. The glycine-rich regions are blocked. (C) Alignment of the ϕXo *gIII* downstream region with that of the ϕLf. The end of *gIII* and the start of *gVI* are shown.

pIII and ϕXv pre-pIII, respectively. The highest degree of identity was found in the C-terminal 100 aa, about 90.5 % among the three pre-pIIIs. In contrast, only 21.8% and 22.9% identity to the pre-pIIIs of ϕLf and ϕXv, respectively, were found in the N-terminal 100 aa. Computer analysis of the ϕXo pre-pIII predicted an 18-residue signal sequence in the N-terminus and a membrane-anchorage region of 17 residues in the C-terminus. In the central region was a 90-aa sequence rich in glycine, aspartic acid, and histidine (Figure 2B). These are structural features typical of filamentous phage pIIIs (Bross et al., 1988; Davis et al., 1985; Endemann and Model, 1995; Hillet al., 1991; Stengele et al., 1990). It is worth noting that similar to the situation in nucleotide sequence comparison, no homology was found between the ϕXo pre-pIII and the amino acid sequence deduced from the complete sequence of the Cf genome (Kuo et al., 1991).

The N-terminal 18 aa of the deduced ϕXo pre-pIII was highly hydrophobic; it had an arginine at position 3 which was followed by 11 hydrophobic residues and ended with an alanine (Figure 2B). This primary structure in the N-terminus of a protein molecule is typical of a signal sequence (Pugsley, 1993). After cleavage between Ala-18 and Ala-19, a mature protein with a MW of 33,158 would be produced. In the ϕLf and ϕXv pre-pIIIs, signal sequences have also been predicted. These sequences are different from one another in amino acid composition but similar in properties of the residues (Figure 2B).

The ϕLf pIII and ϕXv pIII each possesses a region rich in glycine and aspartic acid (Wen and Tseng, 1996; Lin et al., 1999). The ϕLf protein has the amino acid residues repeating as GD (7 times) and GGGD (11 times), whereas the ϕXv protein possesses the repeats appearing as GD (11 times), GGSD (5 times), and GGGD (4 times). As shown in Figure 2B, the central region in the deduced ϕXo pIII was 90-aa long, containing 38 glycine, 29 aspartic acid, and 19 histidine residues. They were clustered mainly as GD, GDGH and GDDH, repeating for 2, 12 and 7 times, respectively, between aa 156-245 (Figure 2B). This region was longer than the corresponding regions of ϕLf pIII (70 residues) and ϕXv pIII (75 residues) (Lin et al., 1999; Wen and Tseng, 1996). Comparison of the three central regions in amino acid composition indicated that the ϕLf pIII contains only glycine and aspartic acid, whereas in addition to these amino acids, the ϕXv protein contains serine (5 residues) and the ϕXo protein contains histidine. Alignment of the three pIIIs revealed that the insertion of three consecutive GDDHs caused the ϕXo central region to be longer than the corresponding regions in the other pIIIs (Figure 2B). In hydropathy plot, the glycine-rich region of the ϕXo pIII is strongly hydrophilic, similar to the property of the glycin-rich regions in the ϕLf and ϕXv pIIIs but a little stronger in hydrophilicity (Figure 3). This is consistent with the higher content of the charged amino acid residues in the ϕXo pIII central region than in the other pIIIs .

The predicted membrane-embedding domain at C-terminus was 17-residue long spanning aa 315 to 331 in the deduced ϕXo pre-pIII (Figure 2B). A similar domain has also been predicted for ϕLf pIII (aa 308 to 324) and ϕXv pIII (aa 303 to 319) (Lin et al., 1999; Figure 2B), presumably required for anchoring the pIII proteins into the inner membrane of host cell. Within these domains, ϕLf and ϕXv have the same sequence, and only one amino acid residue was found to be different in the ϕXo pIII (Figure 2B).

The pIIIs of Ff and IKe possess a very low degree of overall homology (15%), with the highest homology (43%) being found in the regions required for penetration and the non-homologous regions being essential for receptor recognition to recognize and bind specifically to their respective receptor pilus. Therefore, in these two phages, one pIII can not replace the functionally analogous protein of the other phage (Bross et al., 1988; Endemann et al., 1992; Endemann et al., 1993). In contrast to these cases, the deduced pIIIs of ϕLf, ϕXv, and ϕXo possess a high degree of identity. In addition, the three pIIIs are interchangeable (Liu et al., 1998; Lin et al., 1999; see below). These findings suggest that these three phages share the same sequence information required for assembling the pIII

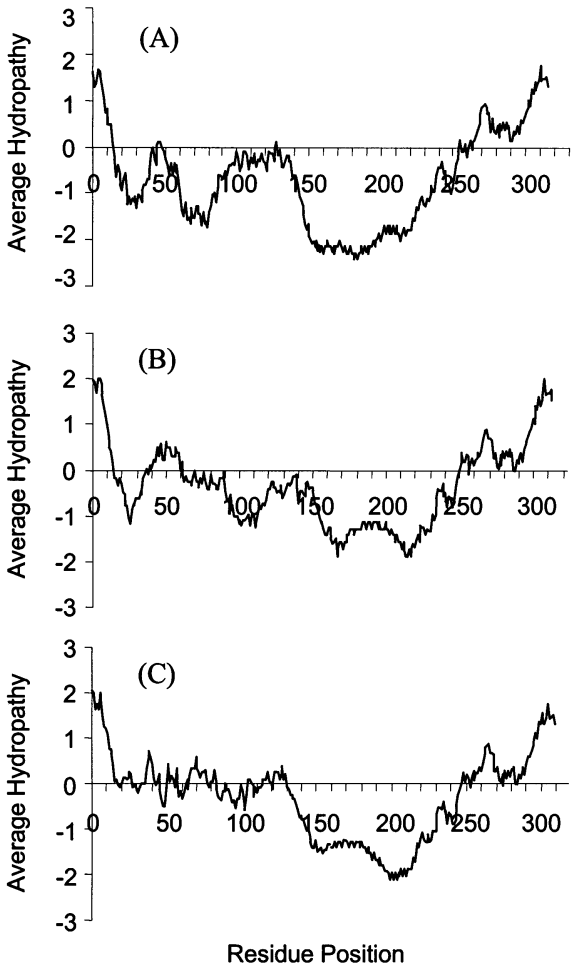

Figure 3. Hydropathy plots for the deduced pIIIs of ϕXo (A), ϕLf (B) and ϕXv (C). A window of 21 amino acids was used with the hydrophobicity scales of Kyte and Doolittle (1982).

into phage particles. The required sequence information in pIII is most likely located in the C-terminus of the polypeptide, since the highest degree of identity is concentrated in this region. On the other hand, to hold restrictive host specificity, specific sequences must be required for receptor recognition and binding. Thus, with a low degree of homology, the N-terminal regions of ca. 100 aa of the three *Xanthomonas* phage pIIIs are the most probable candidates for the receptor recognition.

Purification and Western Blot Analysis of pIIIs

The ϕXo pIII was purified from the viral particles by two passages through the gel filtration column (Suprose 12) in fast protein liquid chromatography (FPLC) as described for the purification of the ϕLf and ϕXv pIIIs (Liu et al., 1998; Lin et al., 1999). The same peak patterns as those in the purification of the ϕLf and ϕXv pIIIs were observed, and the ϕXo pIII was recovered from the second peak from the second chromatography (data not shown). In SDS-PAGE, a single band with a molecular mass of ca. 42 kDa was visualized upon staining the proteins with Coomassie brilliant blue (data not shown). This size was substantially larger than the value calculated for the mature ϕXo pIII (322 residues, 33,158 Da), deduced from the nucleotide sequence determined in this study. Running in the same gel, the ϕLf and ϕXv pIIIs exhibited the same mobility corresponding to a molecular size of ca. 36 kDa, which is slightly larger than the sizes deduced for the mature pIIIs of ϕLf (311 aa, 30,497 Da) and ϕXv (304 aa, 29,463 Da) (Lin et al., 1999; Figure 4). A similar discrepancy has also been observed in Ff phages, in which the mature pIII (424 aa) with a calculated MW of 42,675 exhibits a mobility corresponding to sizes of 59-70 kDa in SDS-PAGE (Endemann and Model, 1995; Goldsmith and Konigsberg, 1977; Woolford et al., 1977). Several explanations have been proposed, one of which attributes the discrepancy to the presence of unusual clustering of glycine and serine in the protein molecule (Endemann and Model, 1995; Woolford et al., 1977). This proposal seems to explain the discrepancy observed in pIIIs of the *Xanthomonas* phages. In addition, the stronger effect on the discrepancy observed in ϕXo pIII than in the pIIIs of ϕLf and ϕXv, presumably due to the presence of three more GDDH repeats, may give further support to this proposal.

It has been shown that the antiserum specific to ϕLf pIII can cross-react with the ϕXv pIII (Lin et al., 1999). In this study, the purified ϕXo pIII was electrophoresed in SDS-polyacrylamide gel, then transferred onto a nylon membrane and subjected to Western blot analysis using the same antiserum, with the pIIIs of ϕLf and ϕXv as the controls. As shown in Figure 4, each of the pIIIs had the same mobility in the SDS-polyacrylamide gel as that described above, and the anti-ϕLf pIII serum was able to cross-react with ϕXo pIII. These results indicate that a high degree of identity in amino acid sequence is shared not only between ϕLf and ϕXv pIIIs, but also between ϕLf and ϕXo pIIIs.

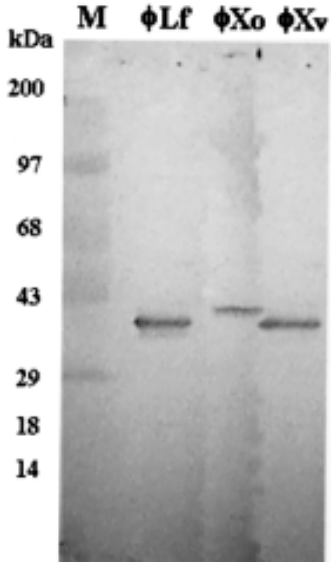

Figure 4. Western blot analysis of the pIIIs from ϕLf, ϕXo and ϕXv. The pIIIs were purified by FPLC, subjected to SDS-polyacrylamide gel electrophoresis, transferred onto nylon membrane and reacted with the antiserum raised against the FPLC-purified ϕLf pIII.

Interchangeability of pIIIs

We have previously demonstrated that the ϕXv and ϕLf pIIIs are interchangeable (Liu et al., 1998; Lin et al., 1999). In this study, we electroporated the ϕXo RF DNA into *X. campestris* pv. campestris strain P20H carrying pRKG3, a plasmid containing cloned ϕLf *gIII*. The results showed that the culture supernatant contained a mixture of authentic ϕXo, which could infect Xo21, and a chimeric phage which could infect P20H. These results indicated that the pIIIs of ϕLf and ϕXo are also interchangeable.

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水稻白葉枯病菌線狀噬菌體 ϕXo 之吸附蛋白

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ϕXo、Xf、ϕLf、ϕXv 與 Cf 為在台灣分離之線狀噬菌體。ϕXo 與 Xf 專一感染 *Xanthomonas oryzae* pv. oryzae，ϕLf、ϕXv 與 Xf 則分別專一感染 *X. campestris* pv. campestris、*X. campestris* pv. vesicatoria 與 *X. campestris* pv. citri。本研究以 ϕLf 之基因 III (*gIII*)（主導吸附蛋白 pIII）為探子選殖到 ϕXv 之 *gIII*。核酸序列分析之結果顯示該 *gIII* 全長 1,023 nt，主導一個 340 個胺基酸（分子量 35,337）之前蛋白。該蛋白具有線狀噬菌體吸附蛋白之特徵：在 N-端有一信號序列（18 個胺基酸），在中段（90 個胺基酸）有高含量的甘胺酸（38 個），天門冬胺酸（29 個）及組胺酸（19 個），及在 C-端有一個供嵌入細胞膜的斗門（17 個胺基酸）。由噬菌體 ϕXo 顆粒純化出來的 pIII 在 SDS-聚丙烯胺凝膠電泳時，泳動的距離與 42 kDa 蛋白之泳動情形相當，而遠大於經由 *gIII* 所推算的分子量。此一情形可能與 ϕXo pIII 的中段含有高量的帶電荷胺基酸基群有關。純化之 ϕXo pIII 能與抗 ϕLf pIII 血清起交叉反應。ϕXo 之 pIII 能與 ϕLf 相交換納入噬菌體顆粒，情形與 ϕLf 的 pIII 與 ϕXv 的 pIII 能相互交換類似。在基因體大小方面，ϕXo (7.6 kb) 與 Xf (7.4 kb) 兩者相似，但比 ϕLf (6.0 kb) 與 ϕXv (6.4 kb) 之基因體都大；不過，ϕXo、ϕLf 與 ϕXv 的 *gIII* 及上下游邊界的段落不僅基因組成相同，長短與核酸序列也都高度相似。此一事實顯示，ϕXo 可能比 ϕLf 及 ϕXv 帶有更多基因，及 / 或 ϕXo 在基因體的其他部分可能有較長的基因間區域。由於 Xf 的 pIII 長達 448 個胺基酸，可見 ϕXo 與 Xf 為 *X. oryzae* pv. oryzae 的兩種不同線狀噬菌體。

關鍵詞：吸附蛋白；天門冬胺酸與組胺酸；基因Ⅲ；高含量之甘胺酸；嵌入細胞膜的斗門；信號序列。