行政院國家科學委員會專題研究計畫　期中進度報告

全基因體化分析克雷白氏肺炎桿菌纖毛基因組的表現(1/3)

計畫類別：個別型計畫
計畫編號：NSC94-3112-B-009-004-
執行期間：94 年 05 月 01 日至 95 年 04 月 30 日
執行單位：國立交通大學生物科技學系(所)

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報告類型：完整報告
報告附件：出席國際會議研究心得報告及發表論文
處理方式：本計畫可公開查詢

中　華　民　國 95 年 5 月 9 日

行政院國家科學委員會補助專題研究計畫 ■ 成 果 報 告
□期中進度報告

Genome wide analysis of expression of the fimbrial operons in *Klebsiella pneumoniae*

計畫類別：■ 個別型計畫　□ 整合型計畫
計畫編號：NSC 94－3112－B－009－004－
執行期間：　九十五 年 五 月 一 日 至 九十六 年 四 月 三十 日

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成果報告類型(依經費核定清單規定繳交)：□精簡報告　■完整報告

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中　華　民　國　九十五 年　四　月　三十 日

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I. Abstract
Ia. Abstract in Chinese

關鍵詞：克雷白氏肺炎桿菌，纖毛，黏附因子，黏附特異性

在克雷白氏肺炎桿菌 NTUH K-2044 基因體中，除了已知的第一型及第三型纖毛外，我們另外發現了七套未被發表過的纖毛基因組，分別命名為 *kpa*，*kpb*，*kpc*，*kpd*，*kpe*，*kpf* 和 *kpg*。為了了解這九套纖毛基因組在黏附特異性及表現調控上的異同，我們在過去一年完成了下列工作：

1. 以 ClustalW 工具分別進行纖毛蛋白質的序列分析，可發現這九套纖毛之間並沒有明顯的親緣關係。

2. 纖毛基因存在分佈率的研究結果顯示，不同來源的123株克雷白氏肺炎桿菌分離株，大多都含有*kpa*、*kpe*、*kpg*、*fim*， 以及*mrk*纖毛基因組 (87% to 100%)。此外，在35株K1莢膜血清型分離株中，有32株具有相同的纖毛基因組合。 然而，這九套纖毛基因組的存在率與疾病種類並沒有明顯的關聯性。

3. 為了瞭解克雷白氏肺炎桿菌調控如何這九種纖毛的表現，我們已分別將這九套纖毛基因子的起使子轉殖到LacZ通報系統，以及製備了九種Balb/c小鼠抗體，可分別偵測九種纖毛的主要結構蛋白質。

4. (1) 九套纖毛基因組已分別自克雷白氏肺炎桿菌 NTUH K-2044中選殖出來，並且以不會生成纖毛的大腸桿菌為宿主，分別表現於大腸桿菌表面。(2) 我們證明第一型及第三型纖毛的表現有互相拮抗的效應 (3) MrkD黏附因子基因序列差異會影響第三型纖毛的生成和特異性表現 (4) 我們證明了MrkF為第三型纖毛的結構蛋白質之一。

I. Abstract
Ib. Abstract in English

Keywords：*Klebsiella pneumoniae*, fimbriae, ahesins, adherence specificities

In the genome of *Klebsiella pneumoniae* NTUH K-2044, in addition to the identified type 1 and type 3 fimbriae, the seven novel fimbrial gene clusters were named *kpa*, *kpb*, *kpc*, *kpd*, *kpe*, *kpf*, and *kpg*. To understand whether these fimbrial operons function synergistically or are regulated differentially, we have accomplished several tasks in the past year as followings:

1. Multiple sequence alignment of the fimbrial genes by ClustalW revealed that the four major components of the nine fimbrial operons are distinct to each other.

2. The prevalence analysis of the nine fimbrial gene clusters among 123 *K. pneumoniae* clinical isolates from various types of disease indicated that *kpa*, *kpe*, *kpg*, *fim*, and *mrk* genes were contained in most of the isolates (87% to 100%). In addition, 32 of 35 K1 isolates appeared to possess identical repertoire of fimbrial operons. However, no obvious correlation between the fimbrial types and diseases could be identified.

3. To characterize each of the fimbriae and investigate if these fimbriae express differentially in the bacteria, each of the putative promoters has been cloned respectively into a LacZ reporter vector and polyclonal antibodies targeting to the major subunit of each of the fimbriae have also been raised from Balb/c mice.

4. (1) Each of the fimbrial operons have been isolated by PCR-based cloning from *K. pneumoniae* NTUH K-2044 and the fimbriae displayed on the surface of an afimbriated *E. coli*. (2) A cross-talk regulation between type1 and type 3 fimbriae has been demonstrated. (3) Allelic variation of *mrkD* adhesin affected fimbriation and activity of type 3 fimbriae (4) MrkF was demonstrated to be a component of type 3 fimbriae.

II. Background and Significance

The biosynthesis and binding properties of type 1 fimbriae have been well studied in *Escherichia coli* (12, 13, 14, 15). Although with less information, type 1 fimbriae have also been isolated and characterized in *K. pneumoniae* (4, 7, 8). Type 3 fimbrial adhesin, referred to as mannose-resistant *Klebsiella*-like (MR/K) hemagglutinin, has been shown to be produced by a few of *K. pneumoniae* strains (1, 8, 9). In addition to the two fimbrial adhesins, three more including the fimbrial antigen KPF-28, the afimbrial adhesin CF29K, and an afimbrial adhesin composed of capsule-like extracellular material have recently been reported in *Klebsiella* isolates (2, 3, 6).

Adhesin specificity plays a major role in determining the host range and tissue tropism in bacterial infection (17). Depending on the expression of type 1 or P fimbriae, UPEC (uropathogenic *E. coli*) vary significantly in their abilities to colonize and persist within the bladder or kidneys (22, 23). PapB, a regulator for phase variation of P fimbriae, and its functional homologue, SfaB of S fimbriae, both were shown to be able to increase the frequency of ON-to-OFF phase for type 1 fimbriae (10). This indicated the presence of a regulatory network in controlling differential expression of type 1-, P-, and S-fimbriae in UPEC. In *E. coli*, type 1 fimbriae has been demonstrated to be essential for the bacteria in establishment and persistence of urinary tract infections (12, 22). Most likely, the fimbriae are also required for *K. pneumoniae* to colonize urinary tract. In addition to uroepithelial cells, the adherence to respiratory epithelia, intestinal and endothelial cells of the *K. pneumoniae* strains expressing type 3 fimbriae have been demonstrated (16). However, the specificity and regulation of fimbrial adherence in *K. pneumoniae* are largely unknown.

Prevalence analysis of *Salmonella enterica* sp. Typhi isolates revealed a unique repertoire of fimbrial gene clusters possibly due to specific selective pressures (20). Expression analysis using flow cytometry of eleven *S.* Typhimurium fimbrial operons indicated further that *in vivo* growth conditions drastically alter the

expression of repertoire of fimbrial antigens (11). Thus, differential expression of the nine fimbriae for *K. pneumoniae* to adhere to different cells or abiotic surfaces, or for the bacterial self-aggregation or biofilm formation could be foreseen. Using bioinformatic analysis, nine sets of fimbriae encoding operons of the chaperone-usher assembly pathway which includes type 1-, type 3- and seven novel-type fimbriae, namely *kpa*, *kpb*, *kpc*, *kpd*, *kpe*, *kpf*, and *kpg* in the genome of *K. pneumoniae* NTUH K-2044 (http://genome.nhri.org.tw/kp/index.php), a K1 isolate of pyogenic liver abscess (5), were identified. The specific aims proposed to be accomplished included:

(1) Analysis of the fimbrial gene clusters in *K. pneumoniae* using the tools of bioinformatics

(2) Investigation of the prevalence of the fimbrial gene clusters in *K. pneumoniae* clinical isolates

(3) Determination of the regulatory control of expression of the fimbrial operons

(4) Characterization of the adherence specificities displayed by each of the fimbrial adhesins

III. Material and Methods

Bacterial strains and growth condition. *K. pneumoniae* NTUH-K2044, of K1 serotype, was provided by Dr. Jin-Town Wang from National Taiwan University Hospital (5). This is a highly invasive and hypermucous strain orignally isolated from the blood of a 40 year old male patient at suffering from community-acquired primary liver abscess and metastatic meningitis (5). The *K. pneumoniae* NTUH-K2044 genome sequence of about 5.5 Mbp was determined by Dr. Shih-Feng Tsai's group in Division of Molecular and Genomic Medicine, National Health Research Institutes (manuscript in submission, the sequences will be released after the manuscript accepted for publication). Clinical isolates of *K. pneumoniae* used in this study were recovered from different tissue specimens of patients with a variety of infections at the Veteran General Hospital, Taipei, from 1991 to 1998. The strains were identified and serotype determined. The bacteria were grown in Luria-Bertani (LB) broth or agar at 37°C and stored at -80°C before use.

PCR amplification for prevalence analysis. The PCR mixture contained 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM (each) deoxynucleoside triphosphates (dATP, dCTP, dGTP, and dTTP), and 1 U of recombinant *Taq* DNA polymerase (Violet Bioscience Inc.) along with each of the *K. pneumoniae* genomic DNA and the specific primers. The primer pairs designed for the respective adhesin and pilin encoding genes of each fimbrial operons are shown in Table 1. The amplification cycle consisted of an initial 1 min hold at 95°C followed by 35 cycles of 95°C for 1 min, 50°C for 1 min, and 72°C for 1 min, and finally an elongation step for 10 min at 72°C. The amplified PCR product was then analyzed by electrophoresis on a 1% agarose gel.

Antiserum preparation. The coding regions corresponding to FimA and MrkA, the major pilin subunit of type 1 fimbriae and type 3 fimbriae, were amplified by PCR and cloned into expression vector pET30, respectively. The expression plasmid was then transformed into *E. coli* Nova-Blue (DE3) and overexpression of the recombinant pilin was induced by addition of 0.5 mM IPTG (isopropyl-β-*D*-thiogalactopyranoside). The MrkA and FimA proteins fusion with His-tag were then purified using a nickel charged resin (Novagen, Madison, WI). In order to raise antibody, five-week-old female BALB/c mice, purchased from the animal center of National Taiwan University, were immunized intraperitoneally with 5 μg of the purified MrkA or FimA. Ten days later, the mice were immunized again with 5 μg of the MrkA or FimA protein. Finally, the antisera were obtained by intracardic puncture.

Western blot analysis of the expression of type 1 and type 3 fimbriae. The clinical isolates of *K. pneumoniae* were grown overnight in either LB, for optimal expression of type 1 fimbriae, or GCAA (minimal medium supplemented with 1% glycerol and 0.3% casamino acids) broth for optimal expression of type 3 fimbriae. Total cellular proteins of the bacteria were resolved by SDS-PAGE and electrophoretically transferred from the gels onto PVDF membranes (Immobilon^{TM}-P, Millipore). The membranes were then blocked with 5% skim milk at room temperature for 1 h, and washed 3 times with 1 X phosphate-buffered saline (PBS). Subsequently, the membranes were incubated with diluted anti-FimA or anti-MrkA serum at room temperature for 1 h. After 3 washes with 1 X PBS, a 3000-fold diluted alkaline phosphatase-conjugated anti-mouse immunoglobulin G was added and the incubation continued for one more hour. The blot was again washed and the bound antibodies were detected by using the chromogenic reagents

BCIP (5-bromo-4-chloro-3-indolyl phosphate) and NBT (Nitro blue tetrazolium).

Statistical methods. Fisher's exact test was used to determine the statistical significance. Values of $p < 0.0001$ were considered significant.

IV. Progress Report and Discussion

Specific aim 1- Analysis of the fimbrial gene clusters in *K. pneumoniae* using the tools of bioinformatics. Using HMMER to search for the Pfam fimbrial family and BLAST analysis of the *K. pneumoniae* NTUH K-2044 genome, nine distinct putative fimbrial operons including type 1 and type 3, and seven novel types of fimbriae, namely *kpa*, *kpb*, *kpc*, *kpd*, *kpe*, *kpf*, and *kpg* were identified (Fig. 1). Each of the fimbrial operons contains the four essential genes for fimbriae biosynthesis, which are pilin, adhesion, chaperone, and usher encoding genes. Multiple sequence alignment of the fimbrial operons by ClustalW indicated that they share amino acid sequence positivities of 26.5% and 36.4%, respectively, for the genes encoding adhesin and pilin; 49.3% and 55.4% for those encoding usher and chaperone.

It is of interest to note that the *fim* and *mrk* gene clusters, encoding respectively type 1 and 3 fimbriae, are physically linked in the *K. pneumoniae* NTUH-K2044 genome. In between the two operons, two genes encoding putative regulators with amino acid sequence identities of 61% and 65% respectively to *Erwinia chrysanthemi* PecS and PecM, the regulators of virulence control in the bacteria were identified. Since the gene organization was also conserved in the genome of *K. pneumoniae* MGH78578 (http://genome.wustl.edu/tools/blast/), the possibility of cross-talk regulation and regulatory roles of the PecS and PecM homologs for the expression of the two fimbriae has also been investigated (described in the section of specific aim 4).

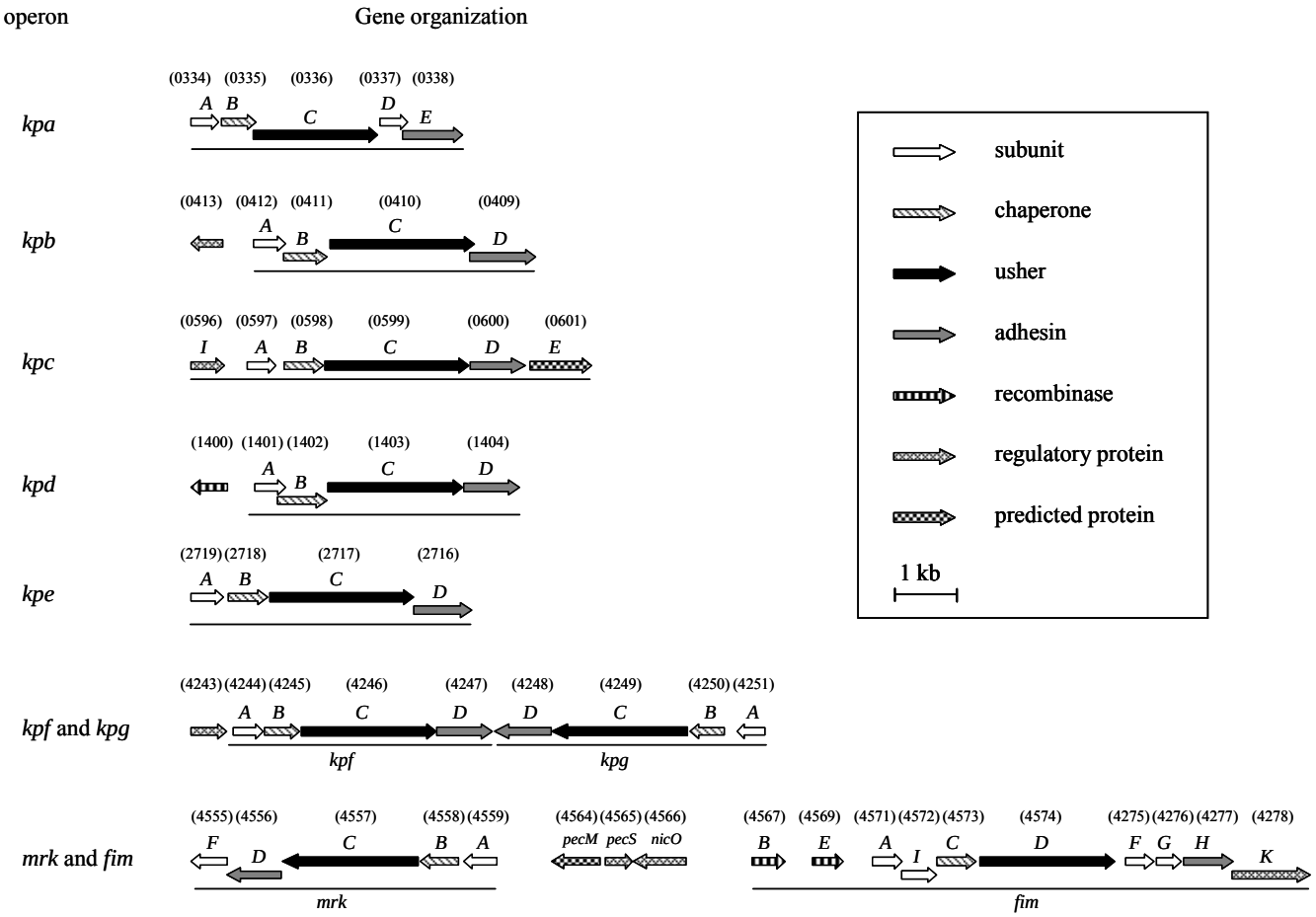


FIG. 1. Fimbrial gene clusters of the chaperone-usher-dependent-dependent assembly class in *K. pneumoniae* NTUH-K2044. The designation of genes and locus tag (KP number) of open reading frames annotated in the *K. pneumoniae* NTUH-K2044 genome were indicated. A total number of nine fimbrial gene clusters and the adjacent regulatory proteins are as shown, and each of the putative fimbrial operons is underlined.

The BLAST analysis also revealed that, except the *kpbABCD* and *kpeABCD* operons, homologs could be identified in other bacteria for *kpa*, *kpc*, *kpd*, *kpg*, and *kpf* gene clusters. The *kpaABCDE* operon, is likely an orthologue of *Salmonella* Typhimurium LT2 *sthABCDE* operon, which has been reported to be required for intestinal persistence in mice. In *kpa* operon, both *kpaA* and *kpaD* genes appeared to be capable of encoding a fimbrial subunit suggesting that they are respectively the major and minor subunits of the fimbrial rod. The *kpcABCD* are homologous to the putative fimbrial genes, plu2159 to plu2156, of *Photorhabdus luminescens* subsp. laumondii TTO1. The *kpdABCD* encodes a homologous operon of Reut02000958 to Reut02000961 in *Ralstonia metallidurans* CH34 (unfinished sequence, GenBank: AAAI00000000). The physically linked *kpfABCD* operon and *kpgABCD* genes appeared to be respectively an orthologue of *Edwardsiella tarda* KG8401 *etfABCD* operon, and an orthologue of the fimbrial operon, plu2159 to plu2156, in *P. luminescens* subsp. laumondii TTO1.

Upstream of the *kpbABCD* operon, a divergently transcribed gene encoding a transcriptional regulator with a DNA-binding domain was identified and designated *kpbR* (Fig. 1). A DNA recombinase encoding gene, namely *kpcI*, located upstream of *kpcABCD*, was found to be an orthologue of several fimbrial invertases including MrpI, MrxI (AAM91928) and MrfI. As shown in Fig. 2, an 11-bp inverted repeat was identified in the putative promoter region of *kpc* operon, impling a phase-variation control by DNA inversion for the operon expression. Moreover, TCS (two component system) response regulator encoding genes, namely *kpdR* and *kpfR*, were found to be located upstream of *kpdABCD* and *kpfABCD* operons (Fig. 1).

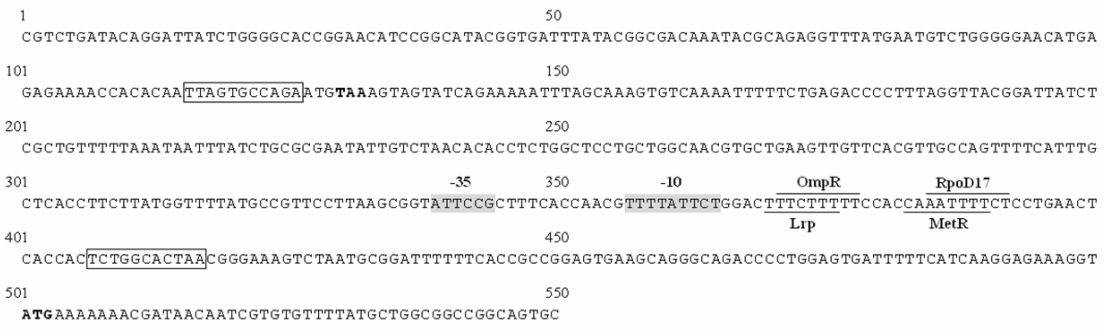


FIG. 2. The DNA sequence of the putative promoter region of *kpc* operon. 500 bp upstream and 50 bp downstream of the *kpcA* translation start codon ATG (bold type) are as shown. The 11 bp inverted repeats are indicated as boxes. The predicted -10 and -35 promoter regions are shadowed. Four predicted transcription factor binding sites are each overlined or underlined. The *kpcI* translation stop codon TAA is also shown as bold type.

Specific aim 2- Investigation of the prevalence of the fimbrial gene clusters in *K. pneumoniae* clinical isolates. Prevalence analysis via PCR detection of the respective pilin and adhesin encoding genes in 123 *K. pneumoniae* clinical isolates from various types of diseases (collected by Dr. CP Fung from the Veteran General Hospital Taipei) revealed that *kpa*, *kpe*, *kpg*, *fim*, and *mrk* genes were contained in most of the isolates (87% to 100%). As shown in Table 1, the distribution of *kpb*, *kpc*, *kpd*, and *kpf* genes were 50%, 32%, 83%, and 70%, respectively. In addition, 32 of 35 K1 isolates appeared to possess identical repertoire of fimbrial operons. No obvious correlation between the fimbrial types and diseases could be identified.

Presence of gene									No. with genes (%)		
kpa	*kpb*	*kpc*	*kpd*	*kpe*	*kpf*	*kpg*	*fim*	*mrk*	K1 serotype	K2 serotype	Other serotypes
(A) Distribution of fimbrial genes in combination with each other among different K serotype bacteria											
+	+	+	+	+	+	+	+	+	32 (91)		6 (7)
+	–	–	+	+	+	+	+	+	1 (3)	1	25 (29)
+	–	–	–	+	+	+	+	+	0		9 (11)
+	+	–	+	+	+	+	+	+	1 (3)		10 (12)
+	–	–	+	+	–	+	+	+	1 (3)		7 (8)
+	–	–	–	–	–	+	–	+	0		6 (7)
+	+	–	+	+	–	+	+	+	0	5	4 (5)
+	–	–	+	+	–	+	–	+	0		2 (2)
+	–	–	+	+	–	+	–	+	0		2 (2)
+	–	–	–	+	–	+	+	+	0	1	1 (1)
+	+	+	+	+	–	+	+	+	0		1 (1)
+	+	–	–	+	+	+	+	+	0		1 (1)
+	+	–	+	+	–	+	–	+	0		1 (1)
+	+	–	–	+	+	+	–	+	0		1 (1)
+	+	–	+	+	–	–	–	+	0		1 (1)
+	–	+	+	+	–	+	–	+	0		1 (1)
–	+	–	–	–	–	+	–	+	0		1 (1)
+	–	–	+	+	+	–	–	+	0		1 (1)
+	–	–	+	+	–	–	–	+	0		1 (1)
(B) No. of isolates (n = 123) presenting specific fimbriae-related DNA sequences (%)											
122 (99)	64 (52)	40 (33)	103 (84)	116 (94)	88 (72)	120 (98)	106 (86)	123 (100)			

TABLE 1. Frequency and repertoire of fimbrial genes among *K. pneumoniae* isolates with different K serotypes

Nevertheless, *kpb* and *kpc* gene clusters were found to be more prevalent ($P < 0.0001$) in the isolates of K1 serotype (Table 2).

(A)

K. pneumoniae isolates	No. of strains/total no. of strains isolated (%)	
	K1 serotype	Other serotypes
Liver abscess	14/14	4/5
Bile		4/9
Urine	1/2	4/14
sputum	5/5	5/15
Wound	6/6	6/18
Blood	7/8	4/18
Ascites		4/9
Total	33/35 (94)*	31/88 (35)*

*, $P < 0.0001$

(B)

K. pneumoniae isolates	No. of strains/total no. of strains isolated (%)	
	K1 serotype	Other serotypes
Liver abscess	14/14	2/5
Bile		1/9
Urine	1/2	0/14
sputum	4/5	1/15
Wound	6/6	1/18
Blood	7/8	2/18
Ascites		1/9
Total	32/35 (91)*	8/88 (11)*

*, $P < 0.0001$

TABLE 2. Prevalence and distribution of *kpb* (A) and *kpc* (B) genes in various isolates of *K. pneumoniae.*

The results indicated that the specific combination and regulation of these fimbrial operons may play important roles in *K. pneumoniae* K1 pathogenesis. Interestingly, western blotting hybridization using anti-FimA and anti-MrkA antibodies, respectively, revealed that only 15.7% and 46.3% of the isolates expressed *in vitro* the major subunit of type 1 and type 3 fimbriae. Nevertheless, type 1 fimbriae were found to be prevalently expressed (71%) in liver abscess isolates of K1 serotype suggesting a not yet identified role of type 1 fimbrial expression for the infection of K1 isolates causing liver abscess (Table 3).

Expression of fimbriae	*K. pneumoniae* isolates (No.)			
	K1 isolates, KLA (14)	K1 isolates, nonKLA (21)	K2 isolates (7)	Other serotypes (81)
% of isolates expressing fimbriae under the specific conditions (No.)				
Type 1 fimbriae	71 (10)	0 (0)	14 (1)	1 (8)
Type 3 fimbriae	14 (2)	10 (2)	43 (3)	62 (50)

TABLE 3. *In vitro* expression of type 1 and type 3 fimbriae

Specific aim 3- Determination of the regulatory control of expression of the fimbrial operons. (1) In order to measure the expression of each of the fimbriae, the putative promoter regions of each of the nine fimbrial gene clusters have been isolated from *K. pneumoniae* NTUH-K2044, and cloned into the reporter plasmid placZ15 containing a promoterless *lacZ* gene, which allow determine the signals that affect the fimbriae expression and the promoter activity of each of the fimbriae (Fig. 3).

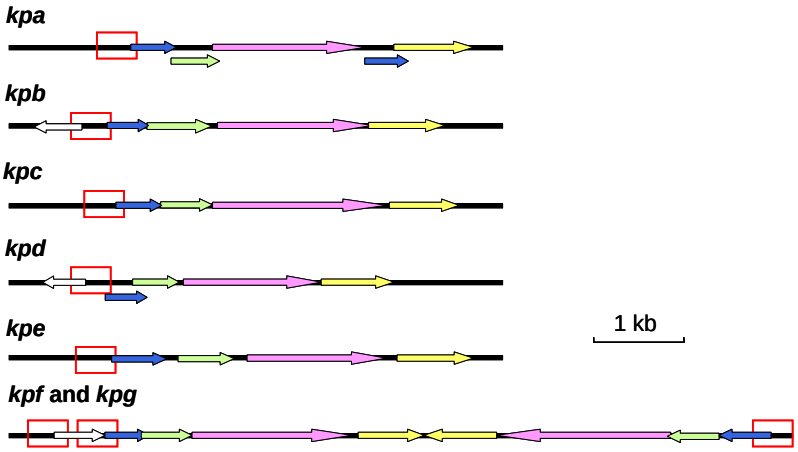


FIG. 3. Schematic diagram of the putative promoters of *kpa*, *kpb*, *kpc*, *kpd*, *kpe*, *kpf*, and *kpg* operons

The activity of the promoters including P-$_{kpa}$, P-$_{kpb}$, P-$_{kpd}$, P-$_{kpf}$, and P-$_{kpg}$ are being analyzed in the presence of different cultural conditions such as changes of nutrient sources, growth temperature, osmolarity, and iron concentration. (2) The heterologous expression constructs in *Escherichia coli* for each of the pilins have been generated, the recombinant proteins purified, and several specific polyclonal antibodies obtained.

Specific aims 4- Characterization of the adherence specificities displayed by each of the fimbrial adhesins. (1) To characterize each of the fimbriae, *E. coli* display systems including *kpd*, *kpe*, *kpf*, *mrk* fimbriae have been obtained and expression of the fimbriae will be confirmed by analysis using both western blotting hybridization and immunofluorescencent microscopy (Fig. 4). The *E. coli* transformants carrying each of the plasmid are being analyzed based on the phenotypic changes including fimbriation activity, autoaggregation, and biofilm formation.

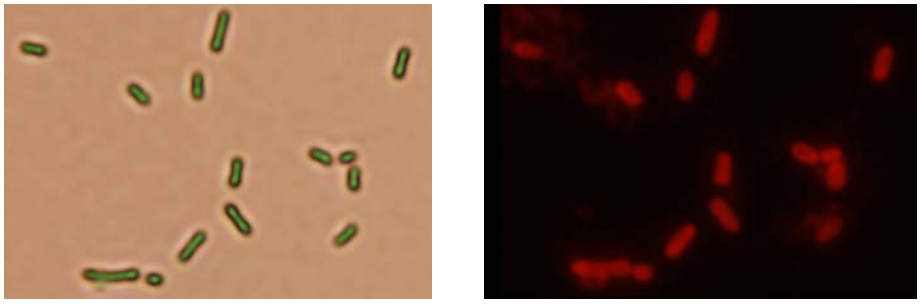


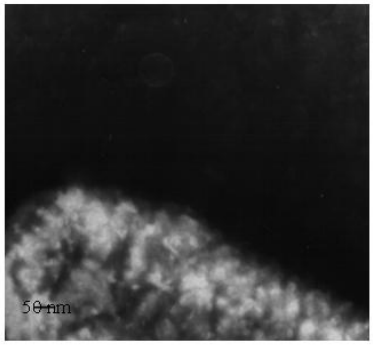
FIG. 4. Kpe fimbriae on the surface of *E. coli* JM109 detected using immunofluorescence microscopy.

(2)-1. As shown in Fig. 1, next to the type 3 fimbriae *mrkABCDF* gene cluster, *orfS* and *orfM* were found following with a *fim* gene cluster encoding the type 1 fimbriae.

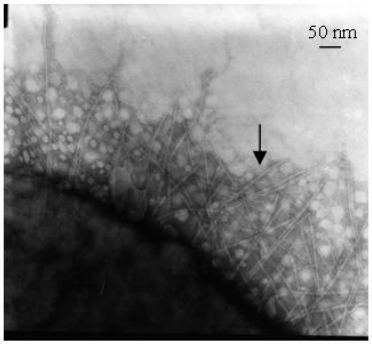
Using either analysis of western blot or immunogold-transmission electronic microscopy (TEM) with anti-MrkA and anti-FimA antisera, the presence of a cross-talk regulation in controlling the expression of type 1 and type 3 fimbriae was demonstrated. In addition, using *lacZ* reporter system and gel mobility shift assay, a regulatory control of the expression of type 3 fimbriae by OrfS/OrfM was determined (manuscript to be submitted).

(2)-2. As shown in Fig. 5, allelic variation of *mrkD* adhesin of type 3 fimbriae apparently influence fimbriation of the variants and also their fimbrial activities assessed using cell adherence assay, biofilm formation capability, and collagen binding assay. Site-directed mutagenesis will be carried out to identify the binding motifs respectively to type IV- and type V-collagen, and to fibronectin.

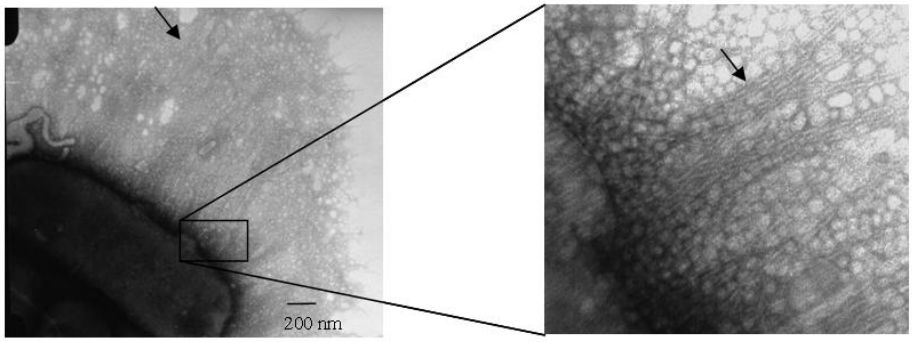
(A) *E. coli* JM109 [pGEM-T]



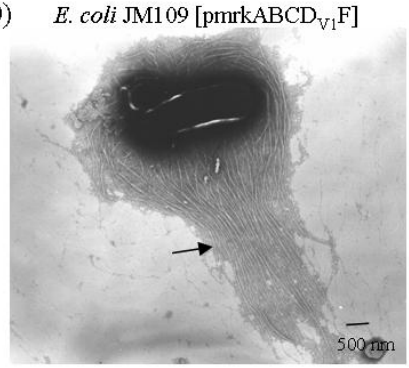
(B) *E. coli* JM109 [pmrkABC]



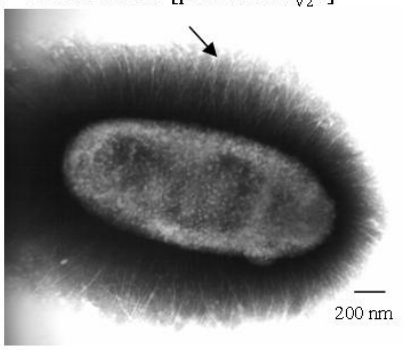
(C) *E. coli* JM109 [pmrkABCF]



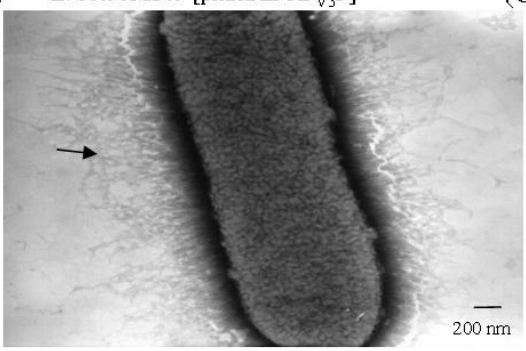
(D) *E. coli* JM109 [pmrkABCD$_{V1}$F]



(E) *E. coli* JM109 [pmrkABCD$_{V2}$F]



(F) *E. coli* JM109 [pmrkABCD$_{V3}$F]



(G) *E. coli* JM109 [pmrkABCD$_{V4}$F]

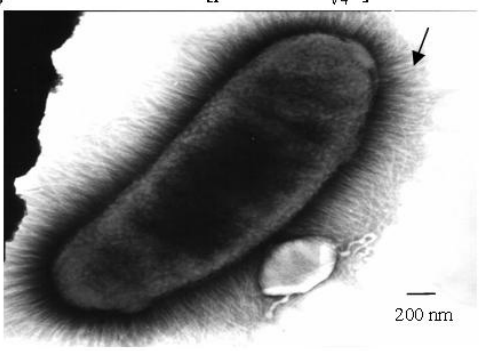


FIG. 5. Transmission electron micrographs of the type 3 fimbriae displaying on *E. coli*. One drop of bacterial suspension was placed on a carbon-coated copper grid, and negatively stained with 2 % phosphotungstic acid. (A) *E. coli* JM109 [pGEM-T], (B) *E. coli* JM109 [pmrkABC], (C) *E. coli* JM109 [pmrkABCF], (D) *E. coli* JM109 [pmrkABCD$_{V1}$F], (E) *E. coli* JM109 [pmrkABCD$_{V2}$F], (F) *E. coli* JM109 [pmrkABCD$_{V3}$F], (G) *E. coli* JM109 [pmrkABCD$_{V4}$F].

(2)-3. Fimbriation analysis of the *E. coli* displaying with various recombinants of type 3 fimbriae including *mrkABC, mrkABCD, mrkABCF, mrkABCDF* using transmission electronic microscopy (Fig. 6), western blotting analysis, and co-immunoprecipitation, demonstrated that MrkF is a component of type 3 fimbriae.

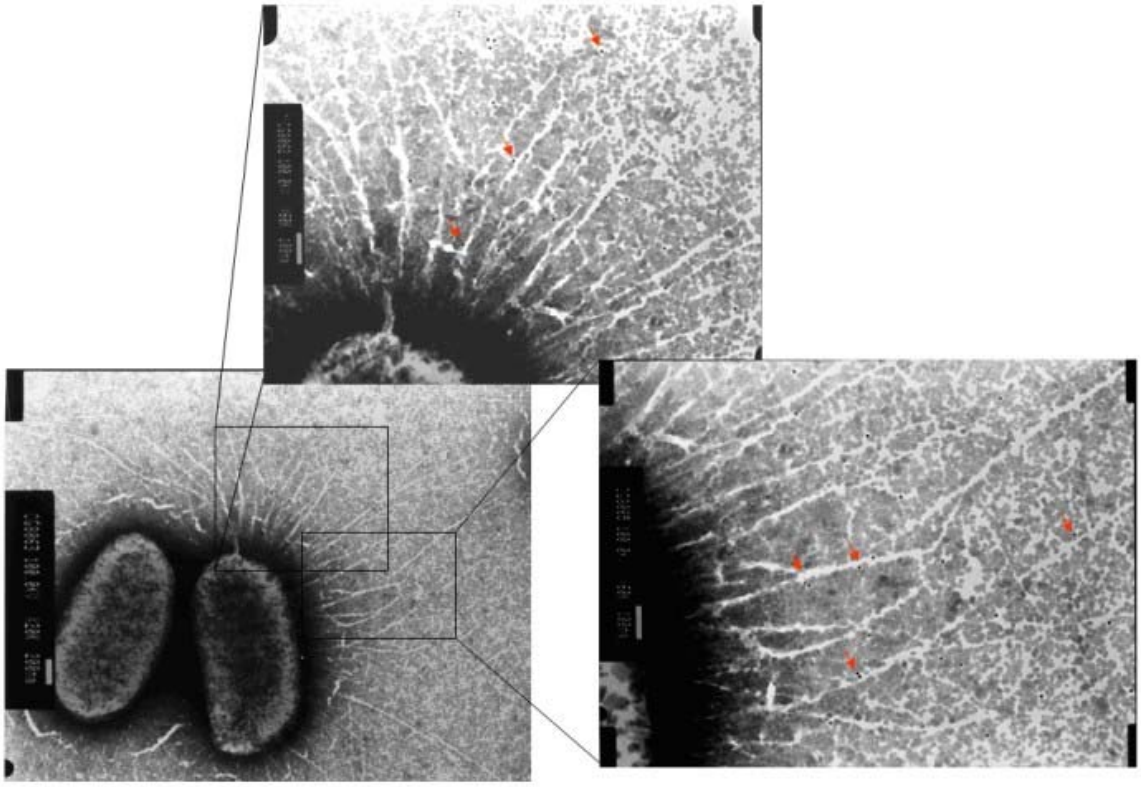


FIG. 6. Type 3 fimbriae labeled using anti-MrkF immuno-gold. The magnified views showed the position of MrkF labeled with 10 nm gold particle.

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VI. Self-Assessment

We have carried out the experiments as planned and several manuscripts (listed as the following) are in the final stage to be submitted for publication. In addition, many interesting findings allow the anticipation of important contributions to unravel the regulation of fimbrial expression at genomic level, the cross-talk regulatory circuit in-between the nine fimbrial gene clusters, and also their roles in infections, which hold promise to provide intervening targets for antimicrobial agents. The manuscripts to be submitted include:

1. **Chien-Chin Wu, Ying-Jung Huang, Chang-Phone Fung, and Hwei-Ling Peng**, Fimbrial gene cluster analysis for characterization of *Klebsiella pneumoniae* clinical isolates.
2. **Ying-Jung Huang and Hwei-Ling Peng**, Regulation of type 3 fimbriae in *Klebsiella pneumoniae* CG43.
3. **Ying-Jung Huang, Chien-Chin Wu, Mei-Chen Chen, Chang-Phone Fung, and Hwei-Ling Peng**, Comparative analysis of the type 3 fimbriae with different *mrkD* adhesin alleles.
4. **Hsin-Wei Liao, Ying-Jung Huang, Chien-Chen Wu, and Hwei-Ling Peng.** MrkF is a component of type 3 fimbriae.