行政院國家科學委員會專題研究計畫 成果報告

探討 Sigma E-dependent small RNAs 在克雷白氏肺炎桿菌的生理適應與致病能力上的調控影響

研究成果報告(精簡版)

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中 文 摘 要 ： 克雷白氏肺炎桿菌 (Klebsiella pneumoniae) 可適應多種環境變化並具有引起多樣化的臨床感染的能力。 究竟 K. pneumoniae 如何藉由不同的生理機制間的轉換以允許其在特定環境中生存仍然是個謎團。 近來許多研究證實在原核細胞適應多樣的環境變化中 Small non-coding RNAs 扮演著重要的調控角色，而有許多 small RNAs (sRNAs) 須藉由 Hfq 的結合在後轉錄的層次上執行他們基因調控的功能。 我們先前的研究發現 hfq 基因缺失造成 K. pneumoniae 喪失細菌致病性。 全基因組 DNA 微陣列分析發現有將近五分之一的 K. pneumoniae 基因(897/5041)的表現量在 hfq 基因缺失的菌株內有顯著改變。 在這 897 個 Hfq-dependent 基因中，13.6% 的基因表現在 Sigma E-knock in 的菌株內有顯著改變。 相對於 Sigma S 缺失的菌株，我們發現 sigma E 的缺失除造成 K. pneumoniae 的毒性喪失外亦會減損其在不同極端環境下的抗壓性。 Sigma E 極有可能是透過 sigma E-driven sRNAs 來協調不同的調控路徑以扮演其在抗壓反應與致病力上多功能的調控角色。有趣的是，兩個 K. pneumoniae 的 sRNAs，RybB 和 MicA 在 Sigma E-knock in 菌株中大量增加其表現量。 雖然 sigma E 是轉錄活化因子，全基因組 DNA 微陣列分析顯示在 K. pneumoniae 有 45%的 Sigma E-dependent 的基因受到負向調控。 由於 RybB 和 MicA 是可以後轉錄抑制基因表現的 RNA 調節子，負向調控 Sigma E-regulon 的基因可能是源自於這 sRNAs 的後轉錄抑制作用。 全基因組 DNA 微陣列分析顯示分別有 31 和 21 個 K. pneumoniae 基因在 RybB 與 MicA 的短時間脈衝表現下，mRNA 的量相較於對照組顯著減少四倍以上，而這些基因與受到大腸桿菌 RybB 與 MicA 調控的基因不盡相同。 藉由探討 RybB 與 MicA 連結 Sigma E-regulon 與其他訊號傳遞路徑的方式，我們對於 small RNAs 如何參與細菌生理適應與致病能力的微調控相關基因表現有初步的了解，而這些結果將可在日後發展新型抗菌藥物上提供有用的資訊。

中文關鍵詞： 克雷白氏肺炎桿菌，微調控 RNA，sigma E

英 文 摘 要 ： Klebsiella pneumoniae adapts itself to various environments and is capable of causing a wild range of infections. How K. pneumoniae switches its physiological programs to ensure survival in a specific niche is still a mystery. Recently, it has become clear that small non-coding RNAs are crucial regulators modulating diverse cellular processes to enable prokaryotic cells to adjust physiological

fitness to environmental changes. A number of regulatory small RNAs (sRNAs) exert their function in the post-transcriptional level via the binding of Hfq. Our previous study demonstrated that the deletion of hfq significantly attenuated K. pneumoniae virulence and drastically deregulated the expression of almost a fifth of K. pneumoniae genes. As Hfq often acts in conjunction with sRNAs, we reason that sRNAs have significant roles in the control of K. pneumoniae gene expression for modulating cellular processes. RybB and MicA, two of K. pneumoniae sRNAs, were strongly activated by the overproduction of sigma E. In contrast to the loss of sigma S, deletion of sigma E-encoding gene rpoE dramatically attenuated K. pneumoniae virulence and abolished its tolerance to diverse stressful conditions. Although sigma E is a transcriptional activator, 45% of sigma E-dependent genes were negatively regulated in K. pneumoniae. Given RybB and MicA are RNA regulators repressing gene expression at the post-transcriptional level; it is possible that the negative regulation of genes belonging to the sigma E-regulon is mediated through the action of sigma E-driven sRNAs. Upon pulse expression of RybB and MicA, DNA microarray analysis revealed that mRNA abundances for 31 and 21 genes, respectively, were significantly decreased with more than 4-fold changes as compared to that of the vector control. The majority of these genes were different from that targeted by E. coli RybB and MicA. The two sRNAs behave like a global regulator but control a distinct set of K. pneumoniae genes from that in E. coli. Through determination of the regulatory mechanism by which RybB and MicA modulate gene expression in K. pneumoniae, we understand how RybB and/or MicA coordinate regulatory networks of stress adaptation and virulence gene expression by linking the 钋E circuit to other signaling pathways. In the near future, the virulence-associated molecules identified in this study may serve as an ideal chemical scaffold for discovery of novel antimicrobial drugs.

英文關鍵詞： Klebsiella pneumoniae，small regulatory RNAs, sigma E

Background and significance

Klebsiella pneumoniae is a Gram-negative enterobacterium ubiquitous in nature. As behaving like a saprophyte resided in nasopharyngeal and intestinal mucosa of humans, *K. pneumoniae* frequently involves in a wide range of clinical illnesses, such as pneumonia, urinary tract infections, suppurative infections, bacteremia, meningitis, and septicemia. Without immediate treatments, infections caused by this bacterium have a significantly high rate of mortality (*1*). During 1990s, *K. pneumoniae* has been noticed as the primary pathogen responsible for community-acquired pyogenic liver abscess (PLA) in Taiwan (*2*). Despite *K. pneumoniae*-caused liver abscess (KLA) was initially thought as a disease of regional distribution, it has now been continually reported from other Asian and Western countries and is considered an emerging disease worldwide (*3*). Distinct from *Escherichia coli*-associated liver abscess, KLA is generically cryptogenic without underlying hepatobiliary disorders and is frequently complicated in up to 10% of cases with septic metastatic lesions to other organs (*2, 4-7*). By virtue of its primary and invasive nature, KLA represents one of the most severe infections caused by *K. pneumoniae* (*8-11*). Not solely confined inside the human host, *K. pneumoniae* has a great capacity to adapt to various environments, including the surface water, sewage, soil, the intestinal tract of other mammals (*1*), and even the interior of plants (*12*). How *K. pneumoniae* responds to environmental changes and thus adapts itself to a specific niche becomes an interesting question. Nevertheless, our knowledge about the regulatory mechanism by which this bacterium switches among different physiological programs to ensure its survival upon different conditions remains incompletely understood.

Post-transcriptional regulations involving small non-coding RNAs (sRNAs) have received considerable attention in recent years (*13*). The bacterial sRNAs, in contrast to the eukaryotic siRNAs and miRNAs, show heterogeneity in size (50~400 nts in length) and structure, and are usually encoded by free-standing genes with a rho-independent terminator (*14*). Different experimental approaches have identified almost a hundred of sRNAs in *Escherichia coli*. For the *E. coli* sRNAs, two main modes of action have been established. While some sRNAs modify the activity of proteins, the majority of sRNAs act through base-pairing with partially complementary sequences in the 5'-untranslated region of trans-encoded target mRNAs to modulate their translation and/or stability (*15*). In many cases, sRNAs that act by the later mechanism do so in complex with the chaperon protein, Hfq. Through assembly as a homohexameric ring, Hfq is structurally similar to the Sm proteins in eukaryotic cells. Except for stabilizing sRNAs and enhancing the formation of sRNA-mRNA pairs to modulate gene expression post-transcriptionally, Hfq can also binds directly to mRNAs to influence messenger stability, polyadenylation, and ribosome binding. Hfq has a broad and diverse impact on bacterial physiology and virulence beyond its original role as a host factor required for replication of Qβ RNA bacteriophage (*16*).

Sigma E (σ^E) is a heat-shock sigma factor discovered in *E. coli* as a positive regulator for sigma H (σ^H) (*17*). The σ^E-encoded gene, *rpoE*, which is located in the *rpoE-rseABC* operon, is essential for *E. coli* growth at extreme

temperatures (*18*). Upon the exposure to extracytoplasmic stress, over 100 *E. coli* genes, including *rpoH* encoding σ^H and *rpoD* encoding σ^D genes (*19*), are known to be expressed by σ^E. To perform such a task, the availability of σ^E is controlled by an ingenious regulatory system. The transcriptional regulator of extracytoplasmic stress, σ^E, is normally held in an inactive complex with the cytoplasmic domains of the anti-sigma factor RseA and RseB (*20*). Events or mutations that lead to the alterations in outer membrane protein (OMP) biogenesis can specifically activate the σ^E response. Signal transduction is mediated by an elegant network of proteolytic cleavages of the anti-sigma factors that is initiated in the periplasm by the DegS protease. Upon external stress, the periplasmic stress sensor DegS becomes activated through recognition of the improperly exposed C-terminal sequences of misfolded OMP precursors by its PDZ domain (*21*). Then, the periplasmic domain of anti-sigma factor RseA is efficiently cleaved by the activated DegS protease. Degradation of RseA leads to the release of σ^E into the cytoplasm and the formation of σ^E–RNA polymerase core complex thereby transcribes a set of genes that includes many that are involved in outer membrane homeostasis.

Until recently, global gene expression studies have mainly been focused on the transcriptional regulation exerted by DNA-binding proteins. With the identification of more and more sRNAs in bacteria, while functions of many of the RNA molecules are still not known, an increasing number of studies demonstrate that the RNA regulators behave as key effectors of bacterial cellular processes. Through rapid post-transcriptional adjustments, the regulatory sRNAs have advantages over protein regulators to rapidly promote bacterial adaptation to ever-changing environments (*22*). Genes subject to post-transcriptional control by sRNAs are involved in numerous cellular processes, such as iron homeostasis (*23*), outer membrane proteins (OMPs) biogenesis (*24*), sugar metabolism (*25*), quorum sensing (*26*) and various stress responses (*27*). Considering the potential impact on coordinating regulatory networks of stress adaptation and virulence gene expression, we pay particular attention on these RNA molecules. A mutant which has in-frame deletion on the gene encoding the RNA chaperone Hfq was therefore generated in *K. pneumoniae* to serve as a starting point for our study on small regulatory RNAs. As shown in C12-01, the deletion of *hfq* significantly attenuated *K. pneumoniae* virulence in the KLA model and drastically deregulated the expression of almost a fifth of *K. pneumoniae* genes (897/5041), as evident by microarray-based transcriptome analyses. As Hfq often acts in conjunction with sRNAs, it is likely that sRNAs play major roles in the control of numerous cellular processes in *K. pneumoniae*.

On the other hand, as it has been shown in *E. coli* that Hfq activities impact the regulation of both the stationary-phase sigma factor S (σ^S) and σ^E (*28*), a significant overlap between Hfq-regulon and σ^E-regulon in *K. pneumoniae* was revealed by our previous study. Of 287 genes that had at least 2.83 fold higher transcript levels in the Δ*hfq* strain, 61 genes were significantly repressed by the overexpression of σ^E. Of 604 genes with decreased expression in the Δ*hfq* strain, the transcripts of 60 genes were significantly up-regulated by the overexpression of σ^E. Overall, 13.6% (121/891) of Hfq-dependent genes belong to the σ^E-regulon. Of particular interest in the findings is the expression for 8 of 39 *K. pneumoniae* sRNA candidates is significantly driven by the overproduction of σ^E. In contrast to the result from a loss of σ^S, the deletion

of σ^E-encoding gene *rpoE* dramatically attenuated *K. pneumoniae* virulence to mice and abolished its tolerance to diverse stressful conditions. The results raise the possibility that σ^E may exert its multi-function through the mediation of sRNAs to coordinate complex pathways for the regulation of stress adaptation and virulence gene expression. To determine the regulatory link of sRNAs to the σ^E regulon, in this project, we will address the roles of the σ^E –dependent sRNAs by characterizing their involvement in the physiology and virulence of *K. pneumoniae*.

Specific aims

Our goal is to determine the roles of σ^E-dependent small RNAs in control of the physiology and virulence of *K. pneumoniae*. The specific aims for the first year are listed as follows.

1. To generate gene-specific deletion mutant for σ^E-dependent sRNAs.
2. To examine bacterial virulence for sRNA deletion mutants.
3. To generate pulse-expressing constructs for virulence-associated sRNA gene.

Results and discussion

1. Hfq downregulates σ^E expression and the deletion of *rpoE* attenuates *K. pneumoniae* virulence. Hfq activities impact the regulation of both the stationary-phase sigma factor RpoS (σ^S) and the envelope stress response sigma factor RpoE (σ^E) (*29, 30*). Our microarray data showed that the transcript level of *rpoS* was down-regulated by 3.6-fold in the absence of *hfq* (Fig. 1A). The decrease of *rpoS* transcripts in Δ*hfq* which was restored to the wild type level by the complementation of *hfq* under the control of pBAD promoter was confirmed by Northern blotting analysis (Fig. 1B). In accordance with this, the protein level of RpoS was down-regulated by the absence of Hfq (Fig. 1C). To determine whether the defects observed in Δ*hfq* were attributed to the defect in RpoS expression, an *rpoS* deletion mutant (*ΔrpoS*) which was generated in the CG43S genetic background was used for comparison with Δ*hfq*. Unlike Δ*hfq*, whose virulence to mice was significantly attenuated, Δ*rpoS* behaved much like CG43S as it caused 80% mortality of mice within one week (open diamonds, Fig. 2). ΔrpoS also displayed wild-type-level tolerance of K.

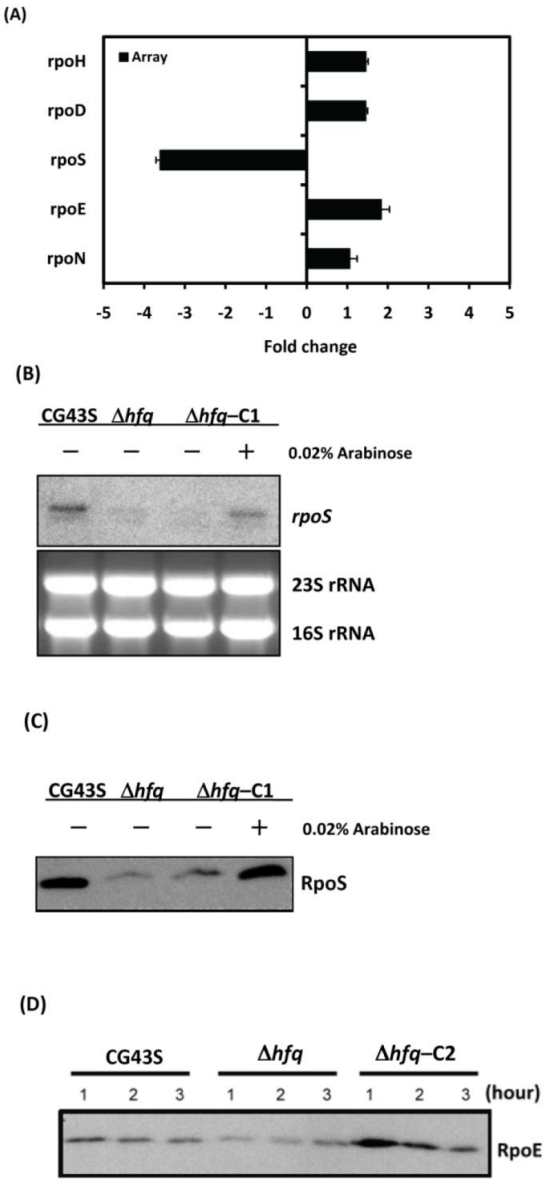

Figure 1. Hfq affected the expression of sigma factors. (A) Fold changes in transcript abundances of *rpoH, rpoD, rpoS, rpoE,* and *rpoN* detected by microarray (black bars) in Δ*hfq* relative to that in CG43S are indicated. **(B)** Transcripts of *rpoS* extracted from CG43S, Δ*hfq*, and Δ*hfq*-C1 were detected by Northern blotting with an *rpoS*-specific biotin-labeled riboprobe. **(C)** RpoS proteins isolated from the LB-grown stationary-phase cultures of CG43S, Δ*hfq*, and Δ*hfq*-C2 with or without the induction of 0.02% arabinose were detected by Western blotting with rabbit anti-RpoS antibody. **(D)** RpoE proteins isolated from the LB-grown cultures of CG43S, Δ*hfq*, and Δ*hfq*-C1 at indicated time points were detected by Western blotting with rabbit anti-RpoE antibody.

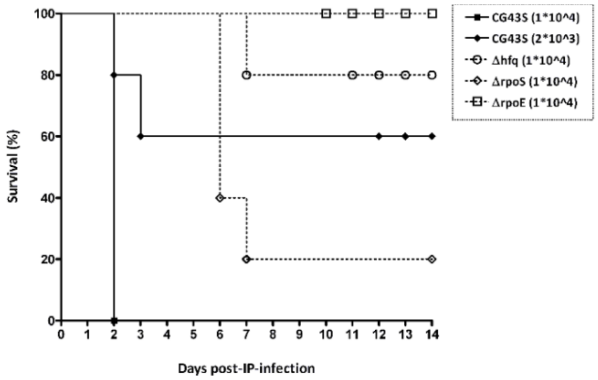

Figure 2. Survival of *K. pneumoniae*-infected mice. Groups of five mice were inoculated by intraperitoneal injection with 2×10^3 CFU of CG43S (filled diamonds), or with 1×10^4 CFU of CG43S (filled squares), Δ*hfq* (open circles), Δ*rpoS* (open diamonds), or Δ*rpoE* (open squares), and monitored for 14 days.

pneumoniae in response to heat shock and UV irradiation (Fig. 3B and C). On the other hand, the loss of rpoS did abolish the ability of K. pneumoniae to conquer H_2O_2 stress (Fig. 3A). The results suggested that the downregulation of rpoS in the Δhfq strain contributed partially to the defects on stress tolerance resulted from the loss of hfq, but could not by itself attenuate the virulence of K. pneumoniae. Meanwhile, the expression of RpoE had also been examined. Although the transcript level of rpoE in the Δhfq strain was found similar to that in CG43S in the microarray analysis, Western blotting analysis revealed that the absence of hfq resulted in decreased protein level of RpoE at early- and mid-log phase (Fig. 1D). An rpoE deletion mutant (ΔrpoE) was generated. Unlike ΔrpoS, ΔrpoE was totally avirulent when given intraperitoneally with the same inoculums that caused 80% mortality in the ΔrpoS-infected group (open squares vs. open diamonds, Fig. 2). ΔrpoE was as sensitive as Δhfq in its responses to heat shock and UV irradiation (Fig. 3B and C), whereas it exhibited a wild-type-level resistance to H_2O_2 (Fig. 3A). These results suggested that the virulence attenuation as well as the loss of tolerance to heat shock and UV irradiation in Δhfq may result from the decrease of RpoE protein by the lack of hfq.

2. Identification of σ^E regulon. Given σ^E contributes to the virulence and stress resistance

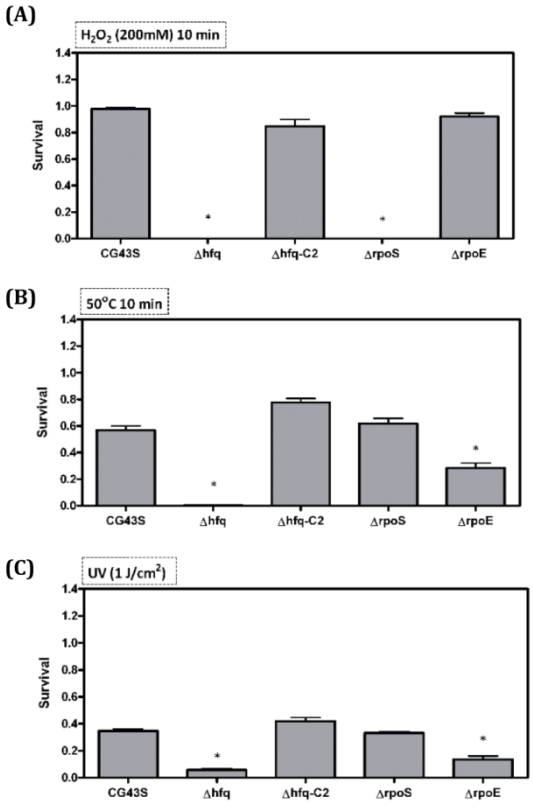

Figure 3. Stress tolerance attenuated in *K. pneumoniae* lacking *hfq*, *rpoS*, or *rpoE*. Survival of *K. pneumoniae* CG43S, Δ*hfq*, Δ*hfq*-C2, Δ*rpoS*, and Δ*rpoE*, upon the treatment of 200 mM H_2O_2 for 10 minutes **(A)**, 50°C shock for 10 minutes **(B)**, or UV irradiation (1 J/cm^2) **(C)** are determined by CFU calculation and presented as (CFU after treatment/CFU before treatment) ×100%. * $P < 0.05$, determined with the Student's *t*-test.

of *K. pneumoniae*, to gain insight into the range of genes with expression that is regulated by σ^E in *K. pneumoniae*, DNA microarrays were performed to compare the transcriptome of *rpoE*-knock-in *K. pneumoniae* with the vector-knock-in control. Probes were made corresponding to RNA transcribed during log-phase growth in LB medium at 37°C. A total of 333 genes (approximately 6.5% of all *K. pneumoniae* genes) had their mRNA abundance increased or decreased with >4-fold change in the *rpoE*-knock-in strain, of which up-regulated genes (n=184) were more than down-regulated genes (n=149). Based on the genome annotation of *K. pneumoniae* NTHU-K2044 (NC012731;(*31*)), these σ^E-dependent genes belong to more than 19 functional categories (Fig. 4). The expression of several categories of genes was notably affected by RpoE. Approximately 11.43% of genes belonging to the class of signal

transduction mechanism were σ^E-dependent [7.62% (8/105) for up-regulated; 3.81% (4/105) for down-regulated). Besides, σ^E-dependency also accounts for 11.06%, 9.02%, and 9.45% of genes belonging to the classes of cell envelope biogenesis, energy production and conversion and inorganic ion transport and metabolism, respectively.

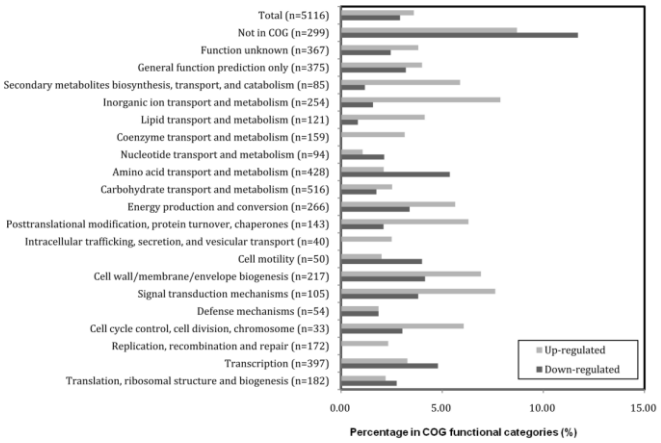

Figure 4. Functional classification of sigma E-dependent genes according to the genome of *K. pneumoniae* NTHU-K2044 (NC012731). The values represent the percentage of genes affected by sigma E in *K. pneumoniae* CG43 within the respective class. Black bars: up-regulated genes; grey bars: down-regulated genes.

3. Identification of σ^E-dependent small RNAs (sRNAs). Bacteria encode a large numbers of sRNAs, most of which have been shown or predicted to act as antisense RNAs on trans-encoded mRNAs. Through translational repression or activation, a number of sRNAs that bind Hfq act as key regulatory elements to modulate multiple aspects of bacterial cell physiology. As *E. coli* is a paradigm for the importance of Hfq in sRNA-based gene regulation, a total of 39 sRNA candidate genes which have homologs and are highly conserved in *E. coli* K-12 were identified in the genome of *K. pneumoniae* NTHU-K2044 (Table 1). To determine whether the impact of *rpoE* deletion on the physiology and virulence of *K. pneumoniae* were attributed to the loss of gene regulation by σ^E-dependent sRNAs, DNA microarrays were performed to compare the transcriptional profiles of the *rpoE*-knock-in strain with vector control for the 39 sRNA candidate genes. As shown in Table 1, 8 sRNAs (Sr0011, Sr0012, Sr0018, Sr0022, Sr0024, Sr0025, Sr0034, and Sr0038) were significantly up-regulated by the expression of σ^E with more than 3-fold difference when compared to the vector control, indicating a direct or indirect dependency of σ^E for the expression of these small RNA genes.

4**. Characterization of *K. pneumoniae* RybB and MicA.** Among the 8 small RNAs, Sr0018 and Sr0034, which are homologues of *E. coli* RybB and MicA, were respectively up-regulated 306-fold and 20-fold by the overproduction of σ^E (Table 1.). Although σ^E is a transcriptional activator, 45% (149/333) of σ^E-dependent genes were negatively regulated in *K. pneumoniae*. Given RybB and MicA are RNA regulators repressing gene expression at the post-transcriptional level; it is possible that the negative regulation of genes belonging to the σ^E-regulon in *K. pneumoniae* is mediated through the action of these σ^E-driven sRNAs. Previous studies of RybB and MicA in *E. coli* and *Salmonella* indicated that these two sRNAs repressed the synthesis of major OMPs by binding in the 5'-mRNA region. More than 20 non-OMP mRNAs were also found to be targeted by *E. coli* RybB and MicA (*32*). These findings suggest that RybB and MicA behave like a global repressor in the post-transcriptional control of *E. coli* gene expression. *K. pneumoniae rybB* and *micA* genes (Fig. 5A) are located (counterclockwise) in the *luxS gshA* and *ybjK* KP1-1841 intergenic regions as previously described in *E. coli* and *S.* Typhimurium (*33, 34*). The sequences of *K. pneumoniae rybB* and *micA* are highly conserved (Fig. 5B). To determine the regulatory impact of RybB and MicA on modulating *K. pneumoniae* gene expression, we performed a genome-wide transcriptome analysis to identify the potential mRNAs that were repressed by the two sRNA regulators. The coding region of *rybB*/*micA* was placed under control of the arabinose inducible araBAD promoter (pBAD202; Invitrogen). The resulting constructs were named pYC458 and pYC463 (Fig. 5C), which were respectively introduced into *K. pneumoniae* to generate the

RybB and MicA knock-in strains. Upon a 10-min pulse-expression of RybB/MicA from the arabinose-inducible plasmid, *K. pneumoniae* genes with changes in mRNA abundance were identified by DNA microarray. As shown in Table 2, by the transient expression of RybB and MicA, a total of 31 and 21 genes, respectively, showed > 4-fold decrease in transcripts levels; of which, 14 genes were shared targets of RybB and MicA. The candidate genes identified in *K. pneumoniae*, except for *ompC*, were different from the set of *E. coli* targets for RybB and/or MicA (*32*). *K. pneumoniae* RybB and MicA have many candidate targets showing no envelope-associated functions, suggesting that the two sRNAs act as a global regulator in *K. pneumoniae*, but control unique sets of genes from that in *E. coli* or *Salmonella*.

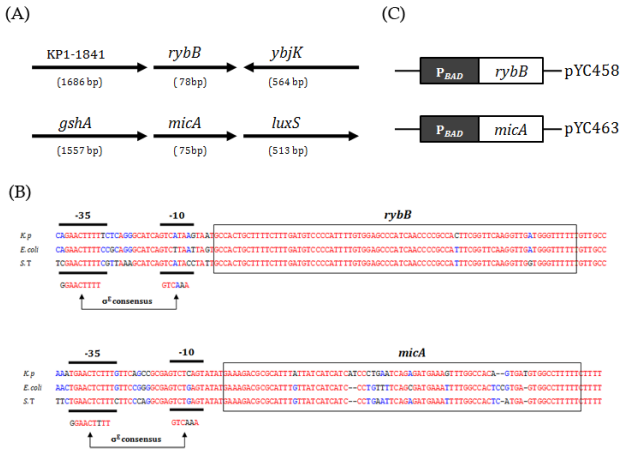

Figure 5. *K. pneumoniae* RybB and MicA. (A) The *K. pneumoniae rybB* and *micA* genes are located (counterclockwise) in the *luxS gshA* and *ybjK* KP1-1841 intergenic regions as previously described in *E. coli* and *S. Typhimurium*. The black arrows indicate the orientation of the genes. **(B)** Conservation of σ^E consensus motifs in the promoters of *rybB and micA genes. K. p: K. pneumoniae, S. T: Salmonella* Typhimurium. The 10/ 35 consensus motifs of σ^E controlled promoters are shown below the alignment. **(C)** Schematic illustration of the arabinose-inducible expression plasmids for *K. pneumoniae rybB* and *micA* genes.

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Table 1. *K. pneumoniae* sRNAs candidates.

No.	Homologues in *E. coli*	Alternative name	Length (bp)	Expression fold change in H0201	Expression fold change by the knock in of RpoE
Sr0004	CsrB		361	1.14±0.20	**-4.19±0.55**
Sr0005	CsrC		246	1.39±0.15	1.25±0.07
Sr0006	DsrA		85	-2.20±0.27	**-3.67±0.14**
Sr0007	ffs		114	-2.48±0.09	1.99±0.15
Sr0008	GcvB		207	**-8.40±0.06**	1.62±0.09
Sr0009	IstR-1		76	-1.48±0.06	-2.27±0.11
Sr0011	MicC		112	1.03±0.12	**7.19±0.18**
Sr0012	MicF		93	**-9.45±0.04**	**6.23±0.03**
Sr0013	OxyS		109	-1.74±0.21	-1.41±0.37
Sr0015	RprA		105	-2.57±0.11	-1.82±0.09
Sr0016	rtT		180	1.87±0.20	-1.74±0.33
Sr0017	RybA		89	2.36±0.10	1.02±0.29
Sr0018	RybB		80	-1.39±0.16	**304.20±0.12**
Sr0019	RydB		68	-2.69±0.12	-1.01±0.08
Sr0020	RyeA	SraC	253	**3.07±0.15**	**-3.21±0.08**
Sr0021	RyeB		124	**-9.71±0.07**	-1.02±0.04
Sr0022	RyeE	CyaR	84	**-14.52±0.09**	**3.66±0.04**
Sr0023	RyfA		338	-1.10±0.15	1.77±0.31
Sr0024	RygA	OmrA	88	-1.16±0.06	**4.37±0.07**
Sr0025	RygB	OmrB	76	**-5.21±0.11**	**5.89±0.06**
Sr0026	RyhA	SraH, ArcZ	115	**-16.11±0.12**	1.89±0.12
Sr0027	RyhB	RhyB	96	-1.22±0.09	2.49±0.04
Sr0028	RyiA	GlmZ	177	**-3.47±0.06**	-2.09±0.12
Sr0029	RyjA		147	-2.18±0.04	-2.63±0.12
Sr0030	SgrS		242	-2.11±0.13	-1.12±0.04
Sr0032	SraA		158	-1.02±0.21	**-3.06±0.31**
Sr0033	SraB		173	**-4.47±0.12**	-2.04±0.06
Sr0034	MicA		77	2.51±0.09	**20.08±0.03**
Sr0035	SraF		188	2.41±0.04	-2.39±0.15
Sr0036	SraG		167	1.22±0.58	**-3.13±0.62**
Sr0037	SroA		100	1.85±0.05	-1.78±0.13
Sr0038	MicM	SroB, RybC	84	**-6.36±0.13**	**3.45±0.10**
Sr0039	SroC		159	**-4.56±0.09**	-1.03±0.10
Sr0040	SroD		87	1.27±0.15	-1.04±0.15
Sr0041	SroE		97	-1.75±0.08	-1.60±0.05
Sr0042	SroF		183	-1.93±0.08	-1.14±0.09
Sr0043	SroG		152	**-6.02±0.16**	-1.60±0.05
Sr0044	SsrA		363	-2.16±0.03	2.61±0.13
Sr0045	SsrS		183	-2.06±0.06	-1.11±0.10

Table 2. RybB/MicA-targeted *K. pneumoniae* genes

Category	Gene/locus	Description	Fold change by the knock-in of RybB		Fold change by the knock-in of MicA	
			Average	STD	Average	STD
Translation	rplA	50S ribosomal protein L1	-5.6	0.4	-4.4	0.2
	rplB	50S ribosomal protein L2			-4.3	0.2
	rpsR	30S ribosomal protein S18			-4.3	0.2
	rpsT	30S ribosomal protein S20	-4.5	0.3		
	rpmI	50S ribosomal protein L35	-4.2	0.1		
	rpsM	30S ribosomal protein S13	-5.0	0.2	-5.1	0.3
	rpsQ	30S ribosomal protein S17	-5.0	0.3	-5.9	0.5
	rplD	50S ribosomal protein L23	-4.8	0.2	-5.5	0.4
	rpsJ	30S ribosomal protein S10	-4.3	0.2	-5.6	0.3
	rpmG	50S ribosomal protein L33	-5.4	0.4	-4.4	0.2
	tsf	elongation factor Ts	-4.5	0.3		
	infA	translation initiation factor IF-1	-5.8	0.2		
Envelope-associated	pulS	pullulanase-specific type II secretion system outer membrane lipoprotein	-4.5	0.3	-4.6	0.6
	KP1_0760	putative PTS permease	-23.5	11.5	-23.3	15.7
	KP1_0761	putative PTS permease	-16.1	4.4	-14.9	4.9
	KP1_0762	putative PTS permease	-27.6	8.1	-10.8	0.6
	KP1_0763	putative PTS permease	-21.2	3.8	-12.7	2.3
	KP1_0764	putative glucosamine-fructose-6-phosphate aminotransferase	-16.1	4.4	-5.4	0.8
	KP1_0765	putative glucosamine-fructose-6-phosphate aminotransferase	-10.0	1.2		
	ompC	outer membrane porin protein C	-5.3	0.3		
	wbbM	putative glycosyltransferase	-4.5	0.1	-5.6	1.2
Metabolism	eutD	cobalamin adenosyltransferase in ethanolamine utilization	-9.6	3.8		
	eutP	putative regulator of ethanolamine utilization	-5.8	0.4		
	eutQ	putative ethanolamine utilization protein	-9.0	3.1		
	suhB	inositol monophosphatase	-4.9	0.6		
	galU	UTP--glucose-1-phosphate uridylyltransferase subunit GalU	-4.5	0.3		
	glf	UDP-galactopyranose mutase	-4.5	0.4		
Other	hdeB	acid-resistance protein	-5.0	0.5		
	hipB	Transcriptional regulator HipB			-4.3	0.3
Unknown	KP1_0176	hypothetical protein			-4.9	0.4
	KP1_1017	hypothetical protein			-5.2	0.8
	KP1_1106	hypothetical protein	-4.8	0.3		
	KP1_1566	hypothetical protein	-4.4	0.3	-4.9	0.4
	KP1_1624	hypothetical protein	-4.4	0.3		
	KP1_2202	hypothetical protein	-5.1	0.6		
	KP1_2314	hypothetical protein			-4.3	0.2
	KP1_2382	hypothetical protein			-6.2	1.4
	KP1_3089	hypothetical protein	-5.1	0.5		

Meeting Report

To gain advanced knowledge in the field of Microbiology, I attend the 102 general meeting of American Society for Microbiology. The meeting was held at San Francisco, CA, USA during 16-19, Jun, 2012. I took a fight on 15, Jun from Taiwan to USA. After the long travel, I arrived at the SFO international airport and then the MOSCON center (Fig. 1), the place for this meeting. This meeting covers various sessions from basic research to diagnostic microbiology, symposia, and more than 3000 posters. This is a big gathering of Microbiologists from USA, Europe, and other countries (Fig. 2-3). Besides, there were more than 1000 booths that exhibited laboratory equipment, research-related reagents, and new techniques from biotech companies (Fig. 4). During a four-day immerse with fascinating talks from outstanding microbiologists oversea, I gathered lots of thoughts regarding my research. Here I highlight several interesting presentations and key concepts that I learned from this meeting.


Fig. 1


Fig. 2


Fig. 3


Fig. 4

(1) The single-cell point of view. It has been pointed that only 40% of proteins are shared among different *E. coli* cells. By the aid of the striking developments in fluorescent microscopy, microfluidics, next-generation sequencing, and mathematical modeling, Dr. Ramunas J. Stepanauskas (Bigelow Lab single cell genomics center for Ocean Sci., West Boothbay Harbor, ME), showed their big progress from single cell genomics (SCG) studies. First, they identified a nanovirus from carnivorous picobiliphytes. Second, they captured macromolecule degraders from megagenomics studies. Third, the SCG resolves genome recovered from the uncultured majority living in the nature. The uncultured majority differs from cultures. Each cell may be unique. For the future of microbiology, I think it is important to analyze and define the characteristics for a single bacterial cell instead of describing the bacterial population from a LB culture.

(2) Microbes trigger and shape immunity and immunity shapes microorganisms. Mucosal surfaces are colonized by microbes that interact with the immune system. Dr. Charles Bevins (Univ. of California Davis) showed his fantastic work regarding the role of innate immune mediators in the host-microbe interaction in the small intestine. Paneath cells of the small intestine play an important role in defending the protrusion of bacteria. They found that HD5 (human defensin 5) was required for the maintenance of microbiota and HD6 had a role in the protection from bacterial dissemination across the intestinal barrier. Dr. Petr Broz's work revealed that NSP1 has a potential to serve as the target for the vaccine development. Caspase 11 mediated cell death leads to increased susceptibility to Salmonella infection in the absence of caspase 1. Taken together, talks from this session mainly focused on mucosal interactions and how microbes promote and alter the development of immune system. As bacteria occupy nearly every niche in ecology, almost every environmental exposed surface of the human/animal body is colonized with bacteria. Therefore, human have developed an essential requirement for association with bacteria, even though bacterial infections adversely affect our health. Adaptive co-evolution has guided this dynamic molecular conservation for millennia. The mechanism employed by pathogenic and symbiotic bacteria to network with the immune system is now being revealed. I learn a lot from these leading experts in this growing field.

(3) Diversity in regulation of gene expression. The tenets of the central dogma of molecular biology were first put forth in the late 1950's, and held that the transfer of genetic information from DNA to protein via RNA was unidirectional. However, recent advances in research revealed that the replication and expression of genetic information can be regulated in myriad ways that fall outside this central dogma. Several interesting talks in this session presented how small non-coding RNA, proteolysis, DNA mobility, and RNA splicing regulate bacterial gene expression. Dr. Jorg Vogel (Univ. Wurzburg, German) used *Salmonella* as a model and found that almost 1% of Salmonella RNA was subjected to the control of gcvB. How the specificity/fidelity is achieved becomes an interesting question to be elucidated. SgrS has a role in the regulation of phophosugar stress by using conserved seed domains to integrate new targets into regulatory network. SopD is one of the new SgrS targets. Sgr discriminates its different targets by a single hydrogen bond and most interestingly, Sgr binds to the coding region of pldB, which is the upstream gene of yigL. YigL is a phosphatase, which dephospho-phosphorylate the phosphor-sugar. Therefore, Dr. Vogel's work tells us that sRNA activates a cistron at the suboperonic level.

國科會補助計畫衍生研發成果推廣資料表

日期:2012/11/13

國科會補助計畫	計畫名稱: 探討Sigma E-dependent small RNAs在克雷白氏肺炎桿菌的生理適應與致病能力上的調控影響
	計畫主持人: 賴怡琪
	計畫編號: 100-2320-B-040-013- 學門領域: 微生物及免疫學

無研發成果推廣資料

100 年度專題研究計畫研究成果彙整表

計畫主持人：賴怡琪	計畫編號：100-2320-B-040-013-
計畫名稱：探討 Sigma E-dependent small RNAs 在克雷白氏肺炎桿菌的生理適應與致病能力上的調控影響	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	2	2	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	無

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：□已發表 □未發表之文稿 ■撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)

In K. pneumoniae, the deletion of the RNA chaperon Hfq and the deletion of the sigma E factor RpoE results in significant difference on the phenotypes when compared to that observed in other pathogens so far analyzed. Therefore, through the determination of the molecular basis of the RpoE-dependent sRNAs modulation in the physiology fitness and virulence potential of K. pneumoniae, several interesting molecular mechanisms which may be unique in this bacterium will be identified. In particular, the identification and characterization of RybB and MicA-target mRNAs provides us insights into how an opportunistic pathogen as K. pneumoniae adapts itself to the host milieu by rapidly fine-tuning virulence-associated genes by small RNA molecules. In the near future, the virulence-associated small RNA molecules may serve as an ideal chemical scaffold for discovery of novel antimicrobial drugs.