

中文摘要

克雷白氏肺炎桿菌是一種機性的病原菌，我們在一株具有高毒性的菌株CG43中發現兩組相似度高的雙分子調控系統基因組，分別命名為*kvgAS-kvhR*和*kvhAS*。根據序列的分析，KvgS和KvhS是組氨酸激酶可以讓細菌感應外在的環境變化，而KvgA、KvhR以及KvhA則是感應蛋白可以反應由組氨酸激酶所傳達的訊息，進而調節下游基因的表現。為了了解這兩套雙分子調控系統在細菌體內所扮演的角色，本研究在克雷白氏肺炎桿菌中建立了以*lacZ*為報導基因的系統，並構築了一系列突變株：*kvgA*⁻ (AZ18)，*kvhA*⁻ (AhZ01)，*kvhR*⁻ (RZ01)，*kvhA*⁻*kvgA*⁻ (AAh01)，*kvhA*⁻*kvhR*⁻ (AhR01)，*kvgA*⁻*kvhA*⁻*kvhR*⁻ (AAhR01)。根據這一系列突變株的表現型分析，第一群帶有*kvgA*或者*kvhR*的基因突變，和野生株比較起來生長、腹腔注射小鼠的半致死率均升高；相反的，第二群*kvhA*⁻以及*kvhA*⁻*kvhR*⁻突變株在上述的表現型分析則和野生株呈現一樣的結果。我們進一步發現第一群菌體的黏性降低是因為莢膜多醣類含量的減低，經由測試莢膜合成基因組中的三個啟動子活性發現：*kvgA*基因的突變會造成*orf16~17*的啟動子活性下降，而*kvhR*基因的突變則造成三個啟動子的活性都下降。而在營養缺乏的環境下，*kvgS*基因的突變不僅會降低*kvgAS*本身啟動子的活性，同時也會使*kvhAS*啟動子的活性降低。經由截短啟動子做活性測試和電泳膠遲滯實驗，本研究除了證明這兩套訊息傳遞系統可以有交互調控外，還分別在*kvgAS*和*kvhAS*的啟動子區域找到KvgA可能的結合片段。進一步也利用



5'-RACE的實驗確認KvgAS和KvhAS的轉錄起始點，而分別在這兩個啟動子序列的-35 上游都發現有RpoS可能的結合位置。進一步在 *rpoS* 突變株中，發現 *kvgAS* 啟動子的活性會明顯降低，而 *kvhAS* 啟動子的活性反而升高，而截除了RpoS可能結合的序列後，*rpoS* 突變對於 *kvgAS* 或 *kvhAS* 啟動子活性的影響也消失了，顯示這各序列經由RpoS結合後進而調控這兩套基因組的表現。同時，本研究也發現 *kvgA* 的基因缺損會降低 *sodC* 和 *katG* 的啟動子表現，這暗示著KvgAS是RpoS調控網路的一員。而大量表現KvhA，則會造成菌體對fosfomycin的敏感度升高，並使菌體內UDP-*N*-acetylglucosamine enolpyruvyl transferase活性上升，這暗示著KvhA

在細菌對抗生素抗性上扮演
系統、cDNA表現異型分析以及
的目標基因，希望未來進一步
控途徑。



研究分別利用啟動子誘捕系
能受這兩套訊息基因調控組
出這兩套訊息傳遞系統的調

Abstract

Klebsiella pneumoniae is a common opportunistic pathogen. Two-component system (2CS) gene clusters including *kvgAS*, *kvhR* and *kvhAS*, have previously been isolated from a highly virulent strain *K. pneumoniae* CG43. According to sequence analysis, KvgS and KvhS are sensory histidine kinases which allow bacteria to sense the signal changes in their environment. KvgA, KvhA and KvhR are the response regulators responding to the signal relayed by the cognate sensor thereby regulating the downstream genes expression. To identify the functional roles of the 2CSs, a LacZ

reporter system CG43S3-Z01

(AZ18), *kvhA*⁻ (AhZ01), *kvhI*

kvgA⁻*kvhR*⁻ (AR01), and *kvgA*



ived mutants including *kvgA*⁻

ΔAh01), *kvhA*⁻*kvhR*⁻ (AhR01),

were constructed. Comparative

analysis of their growth and phenotype allowed the classification of the mutants into two groups: group I bacteria carrying either *kvgA* or *kvhR* mutation displayed less mucoidy, a faster growth rate and an increase of LD₅₀ by comparing to the parental strain Z01. In contrast, the group II bacteria including *kvhA*⁻ and *kvhA*⁻*kvhR*⁻ mutants exert a similar phenotype with that of the parental strain. Decreasing amount of glucuronic acid, the core component of *Klebsiella* capsule polysaccharide (CPS), was found for the group I mutants. Comparing the promoter activity of the three K2-*cps* transcription units of wild type, AZ18, and RZ01 revealed that deletion of *kvgA*

decreased the promoter activity of *orf16-17*, while deletion of *kvhR* reduced the promoter activities of all three *cps* transcripts. Deletion of *kvgS* appeared to reduce not only the expression of *kvgAS*, but also the expression of *kvhAS* in minimal medium. Both promoter activity measurement and EMSA analysis allowed localization of the binding elements of KvgA on both putative promoters P_{kvgAS} and P_{kvhAS} , which indicating an interacting regulation between the two 2CSs. Using 5'-RACE analysis, both identified promoters of *kvgAS* and *kvhAS* appeared to possess a relatively conserved -10 and -35 sequences for Sigma-70 regulation. In addition, a close-to-consensus RpoS binding site was located upstream of both promoters, suggested an RpoS-dependent regulation of *kvgAS* but *kvhAS* expression, supporting an RpoS-dependent regulation of the two 2CSs. Promoter activity measurement further help to confirm that the RpoS binding sites are contained respectively in the consensus sequences of the two promoters. Moreover, deletion of *kvgA* was shown to affect the expression of the antioxidant defense genes *katG* and *sodC*, which are components of the RpoS regulon, at transcriptional level. Overexpression of KvhA altered the susceptibility of the bacteria to fosfomycin and resulted in an increased activity of UDP-*N*-acetylglucosamine enolpyruvyl transferase, the target protein of fosfomycin. Taken together, these indicated that the two 2CSs probably belong to



different regulatory circuits of the RpoS regulon. Finally, we also employed a promoter trapping system, cDNA subtractive hybridization analysis, and proteomic approach to identify the target sequences under the control of the 2CSs. However, more experiments will need to be carried out to demonstrate the regulation of the 2CSs on the expression of the target genes. Till then, a regulatory network of what could be determined completely.



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從來很老套，也不知道在多少
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Abbreviations

2CS	Two-component system
2D	Two-dimensional
5'-RACE	5'-Rapid amplification of cDNA ends
BCIP	5-bromo-4-chloro-3-indolyl phosphate
BSA	Bovine serum albumin
<i>bvg</i>	<i>Bordetella</i> virulence gene
CFU	Colony forming unit
CPS	Capsule polysaccharide
DEPC	Diethylpyrocarbonate
DTT	Dithiothreitol
EDTA	Ethylenediamine-tetraacetic acid
EMSA	Electrophor
<i>evg</i>	<i>Escherichia</i>
IPTG	Isopropyl-β
<i>kvg</i>	<i>Klebsiella</i> \
<i>kvh</i>	<i>Klebsiella</i> \
LB	Luria-Bertan medium
NBT	Nitro blue tetrazolium
PAGE	Polyacrylamide gel electrophoresis
PEG	Polyethylene glycol
PNK	T4 polynucleotide kinase
PCR	Polymerase Chain Reaction
RLU	Relative light unit
SDS	Sodium dodecyl sulfate

