行政院國家科學委員會專題研究計畫成果報告

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一、中文摘要

為了解豬瘟沙氏桿菌 (*Salmonella choleraesuis*) 之攝鐵機制，本計畫對台灣地區養豬場臨床病例分離之 10 株 *S. choleraesuis*，進行 Chrome azurol S (CAS) 培養基分析，結果顯示在限鐵環境下這些菌株均有產生螯鐵蛋白 (siderophore) 之能力。再將這些菌株進行 bioassay，結果顯示均可產生 catechol 類的螯鐵蛋白，但無法產生 hydroxamate 類的螯鐵蛋白。而利用 Fur Titration Assay (FURTA) 方式，選殖出了 32 個 clones，其中已確定 15 個 clones 之質體具有 Fur-box 同源性序列；8 個 clones 具有攝鐵相關基因。

關鍵詞：豬瘟沙氏桿菌、鐵、螯鐵蛋白、攝鐵基因、鐵調節基因、CAS、FURTA

Abstract

To understand the iron utilizing mechanism of *Salmonella choleraesuis*, we chose ten strains of *S. choleraesuis* isolated from clinically affected swine farms in Taiwan, and proceeded with Chrom Azurol S (CAS) plate assay. The result of CAS plate assay showed that all these strains were available to produce siderophores on condition that iron was restricted. Furthermore, the result of bioassay showed that all the ten isolates could produce catechol siderophores, but not hydroxamate siderophores. Using Fur Titration Assay (FURTA), we found 32 FURTA positive clones. Fifteen plasmids of these clones had Fur-box homologous regions, and eight clones had iron-uptake genes or iron-regulated genes.

Keywords: *Salmonella choleraesuis*, iron, siderophore, iron-uptake genes, iron-regulated genes, CAS, FURTA

二、緣由與目的

病原菌能否從宿主攝取鐵份對其致病能力有極大影響。而探討病原菌以何種方式攝鐵，可幫助了解其毒性表現與致病機制。本計畫目的係欲了解豬瘟沙氏桿菌 (*Salmonella choleraesuis*, SC) 毒性表現之關係。計畫自 1997 年 8 月開始執行，選取 10 株臨床病例分離之 SC 培養在限鐵環境中，投予不同鐵源，進行生長狀況之測定；並作 CAS 試驗，測定其產生螯鐵蛋白 (siderophore) 之能力；同時作 bioassay 試驗，分析其產生 siderophores 之種類；另外以 Fur (ferric uptake regulation) titration assay (FURTA) 來選殖 SC 之攝鐵相關基因，並作基因之定序及分析。

三、結果與討論

CAS 及 Bioassay 分析結果

由台灣地區罹患敗血症型沙氏桿菌症之豬隻所分離之 *Salmonella choleraesuis* 10 株，以 CAS blue agar 檢測其產生

siderophore 的能力。結果所有菌株在此培養基，都會形成橙黃色的暈，顯示能產生 siderophres，因此可從 ternary complex chorme azurol S-Fe(III)-hexadecyltrimethyl-ammonium bromide 這個鐵-染劑複合物中移去鐵。將這些菌株培養在含有 *S. typhimurium* mutants enb-7 的 bioassay 培養基，結果顯示均可產生 catecholate 類的螯鐵蛋白 (enterobactin)。但無法產生 hydroxamate 類的螯鐵蛋白 (aerobactin)。

以 FURTA 方法選殖攝鐵相關基因結果

本研究室已由豬隻臨床分離株 (*Salmonella choleraesuis* No. 1459) 染色體 DNA 構築攜帶 Fur-regulated promoter 片段基因之質體。所有菌落以 FURTA 篩檢，共獲 32 個 FURTA-positive clones，目前完成其中 15 個 clones 之 promoter regions 之定序及基因分析，並用 FURTA 程式軟體比對，顯示具有 Fur-box 同源性序列，詳如 Table 1. 所載。上述 15 個質體中之 pSC2，pSC53，pSC147，pSC9 等四個質體具有 iron-uptake genes；其中 pSC2 具 *fes* 基因，推測具表現 ferric enterochelin esterase 之功能；pSC53 具 *cir* 基因，與表現 catechol 類 siderophore 之 receptors 有關；pSC9 之 *fep B* 基因推測可表現 ferrienterobactin-transport protein；pSC147 之 *fep A* 則可能 encode 了 fungal ferrienterochelin 之 receptors。詳如 Fig 1. 所示。另外 17 個質體序列尚在比對中。由已知結果證明 FURTA 篩檢攝鐵調控基因效率甚高。

四、計畫成果自評

本研究室在進行不同鐵源 SC 生長狀況測定之初，曾遇鐵結合蛋白，如 Transferrin、Lactoferrin 等，需先經部分鐵飽和 (partial iron-saturated) 步驟之問題；今已經計算方式求得困難之解決，不久在此部份將會有完整之結果，並可與以 FURTA 方法選殖攝鐵相關基因之結果相對照比較，如此將對 SC 攝鐵機制可有更深入之瞭解。

五、參考文獻

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Table 1. *Salmonella choleraesuis* sequences containing potential Fur-boxes

plasmid	sequence	FURTA
	GATAATGAT A ATCATTATC	(consensus)
pSC2	GATAACTAT T TGCATTTGC	+++
pSC27	GCAAATGCA A ATAGTTATC	++
pSC33	GATACTGAT T ATGGTTTAT	+++
pSC43	GGTAATTAT T ATCATTCTC	+++
pSC47	GAGAATGAT A ATAATTACC	+++
pSC53	GCAAACAAT A ATAATTATC	++
pSC83	GTTAATCAT A ACCGTGATA	+++
pSC9	AGAAATAAT A AGCATTAAC	+++
pSC97	GAAAATGAG A AGCATTAAC	+++
pSC107	GATAATCAC T ATCATTATC	++
pSC123	GATACTGAT T ATGGTTTAT	+++
pSC147	GCAAATGCA A ATAGTTATC	+++
pSC157	GCTCATGAT T CGTATTTCC	++
pSC153	CTTGAAGAT A ATGGTTACC	+++
pSC163	GATAATGAA A AAGGTTATG	+++

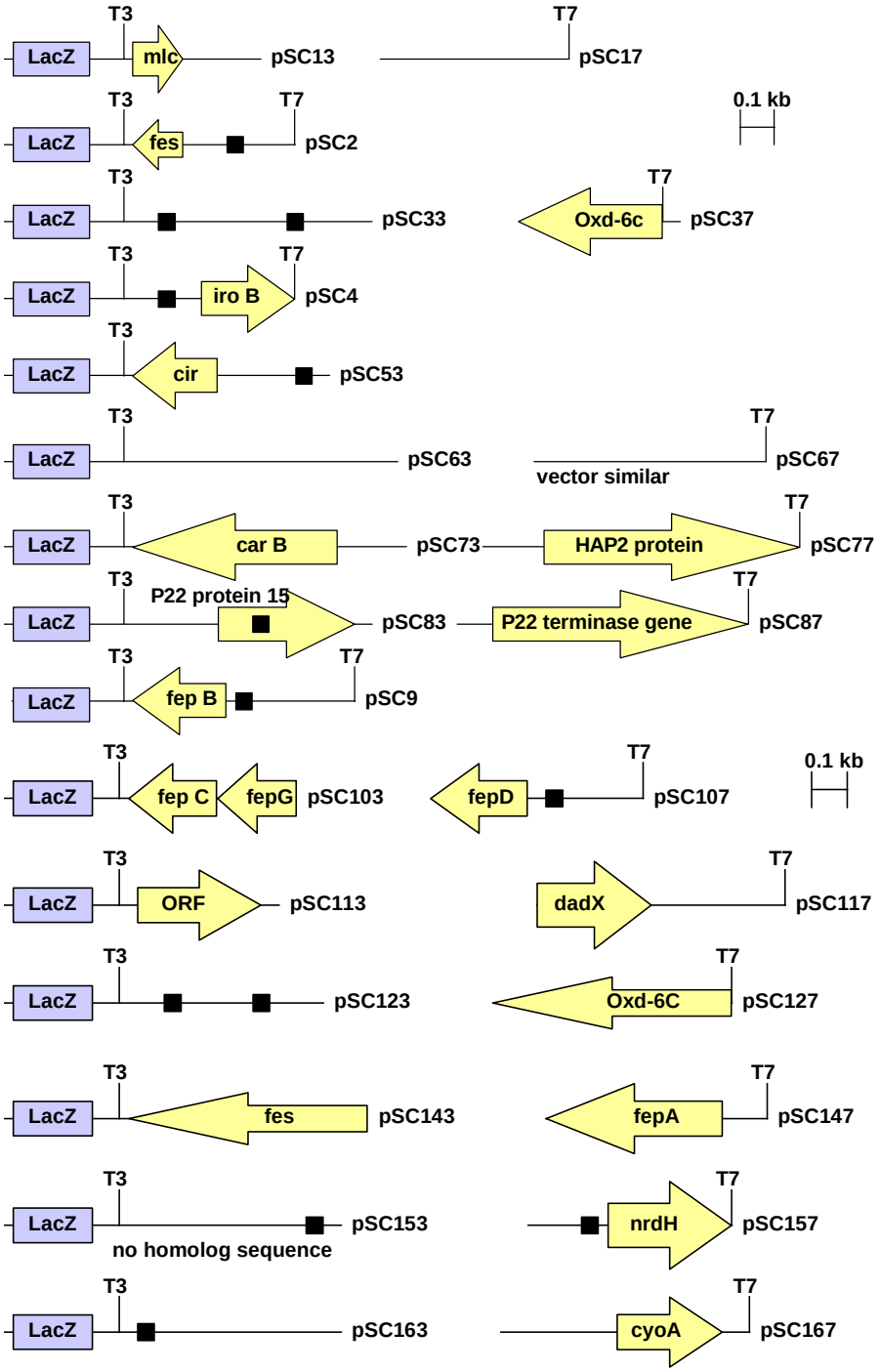

Fig 1. Inserts of FURTA-positive clones which cloned from *Salmonella choleraesuis*. Open arrows indicate open reading frames and the direction of promoters. Black boxes indicate the presence of consensus Fur binding sites. T3: T3 primers used for sequencing. T7: T7 primers used for sequencing. mlc: making-large-colonies protein gene. fes: enterochelin esterase gene. Oxd-6c: transmembrane protein gene. iro B: *Salmonella typhi* glucosyl-transferase homolog gene. cir: colicin I receptor gene. car B: *Salmonella typhimurium* LT2 carbamoyl phosphate synthetase large subunit gene. HAP2 protein: hook-associated protein 2 gene. P22 protein 15: bacteriophage P22 protein 15 gene. P22 terminase gene: bacreriophage P22 terminase (protein 3) gene. fepB: *E. coli* ferrienterobactin transport protein gene. fep C: *E. coli* ferric enterobactin transport protein (ATP-binding protein) gene. fep G: *E. coli* ferric enterobactin transport protein gene. fep D: ferric enterobactin transport protein. ORF: *E. coli* K-12 ORF. dadX: alanine racemase (catabolic precursor) gene. fes: *E. coli* enterochelin esterase gene. fep A: E. coli ferrioenterochelin receptor gene. nrd H: *Salmonella typhimurium* hypothetical glutaredoxin-like 9.1 K protein gene. cyo A: cytochrome o ubiquinol oxidase subunit II gene.