行政院國家科學委員會專題研究計畫成果報告

計畫名稱：*Salmonella choleraesuis*之攝鐵基因

The genes for iron acquisition in *Salmonella choleraesuis*

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一、中文摘要

*Salmonella choleraesuis*是重要之豬細菌性傳染病病原，引起養豬場重大經濟損失。本計畫已由源自*Salmonella*致病島-1 &-2(*Salmonella* pathogenicity island-2; SPI-1 &-2)之相關基因選殖出8個部份基因群落(partial gene clone)。SPI-1 &-2基因源自*Salmonella choleraesuis* & *S. typhimurium*。SPI-1 &-2基因群間有96-98%同質性。現正以對偶基因交換(allelic exchange)技術進行突變株產生．所有突變株以動物試驗進行免疫性及病原性篩選。

Abstract

Salmonella choleraesuis is an important porcine bacterial pathogen, which causes an economic loss in swine industry. We have cloned partial genes derived from *Salmonella* pathogenicity island-1 &-2 (SPI-1 &-2). SPI-1 &-2 genes derived from *Salmonella choleraesuis* and *S. typhimurium* showing approximately 96-98% identity. We are in process to construct SPI-1 &-2 mutant strains by allelic exchange, analyzing the immunological and pathological responses in animals.

Keywords: *Salmonella choleraesuis*, cloning, *Salmonella* pathogenicity island-1 &- 2 (SPI-1 &-2)

二、Introduction

Salmonellosis is of both animal and public health significance. In Taiwan and many other countries, *Salmonella choleraesuis* is the most frequently isolated serotype and is responsible for septicemia in pigs. The pathogenesis of *S. choleraesuis* infection in pigs is still not clear. *S. typhimurium*, which causes a systemic infection in mice known as murine typhoid, encodes several *Salmonella* pathogenicity islands (SPI). SPI-1, located at 63 centisome on chromosome, and SPI-2, located at 30 centisome, belong to the type III secretion system of bacteria. Several Gram-negative bacterial pathogens

secrete virulence proteins via specialized type III secretion systems. SPI-1 controls bacterial invasion of epithelial cells and macrophages, induces the morphological changes and programmed cell death of host cells. SPI-2 plays a crucial role in systemic disease and intramacrophage survival/replication. *S. choleraesuis* also contains SPI-1 and SPI-2, however, their functions in porcine salmonellosis are still unknown. We have cloned the partial genes of both SPI-1 and SPI-2. Further characterization of SPI-1 and SPI-2 is in progress.

三、Results & Discussion

Cloning of SPI-2 and SPI-1

Genomic DNA of *Salmonella choleraesuis* was partially digested by *Sau*3A and the 10-20 kb DNA fragments were purified and cloned into lambda-DASH. Gene derived from *S. typhimurium* by PCR amplification was used as a probe. We have subcloned a 15-kb fragment into pHGKS vector. DNA sequence is still in progress. Based on the partial DNA sequence indicated that the 15 kb fragment contains *ssa*J-*ssa*U region (Fig. 1). The identity of this region between *S. choleraesuis* and *S. typhimurium* is approximately 96-98 % by BLAST comparison.

From our preliminary data and the previous papers reported by others (2,4), the gene sequences of SPI-2 between *S. choleraesuis* and *S. typhimurium* are similar. We also cloned the *spi*C-*spi*B genes, which encode the effector and apparatus proteins of type III secretion system (6), the *sse*A-*sse*C genes, encodes the effector proteins (3), and *hil*A gene, the regulator of SPI-1 (1) (Figure 2). DNA sequencing shows these genes are 93-98 % identity to that of *S. typhimurium*.

Construction of SPI-1 and 2 mutant strains

To create the SPI-2 mutant strains, allelic exchange technique was used. We focus on the *sseA* and *spiC* genes, the effectors of type III secretion system. We cloned the PCR product, which has been deleted partial internal sequences, inserted into a promotorless antibiotic resistence cassette into the PCR product, subcloned this internal deleted gene into the suicide vector pKAS32 (5). These constructs are transfered into *S. choleraesuis* by electroporation.

Further characterization of the SPI-2 mutant strains in the pathogenesis of *S. choleraesuis* in swine, including the virulence (LD_{50}), the cytokine response, and free radical production. These data will help us to understand the role of these gene productions in the disease process.

四、References

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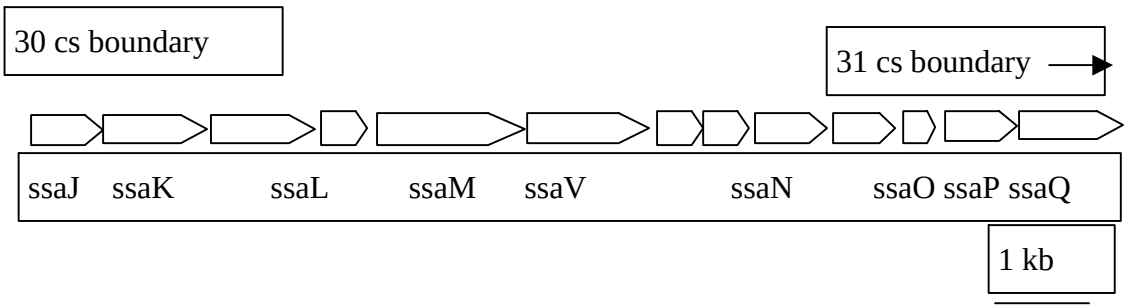

Figure 1: The 15-kb fragment is located at *ssa*J-*ssa*U region of Salmonella pathogenicity island-2. ssa: secretion system apparatus.

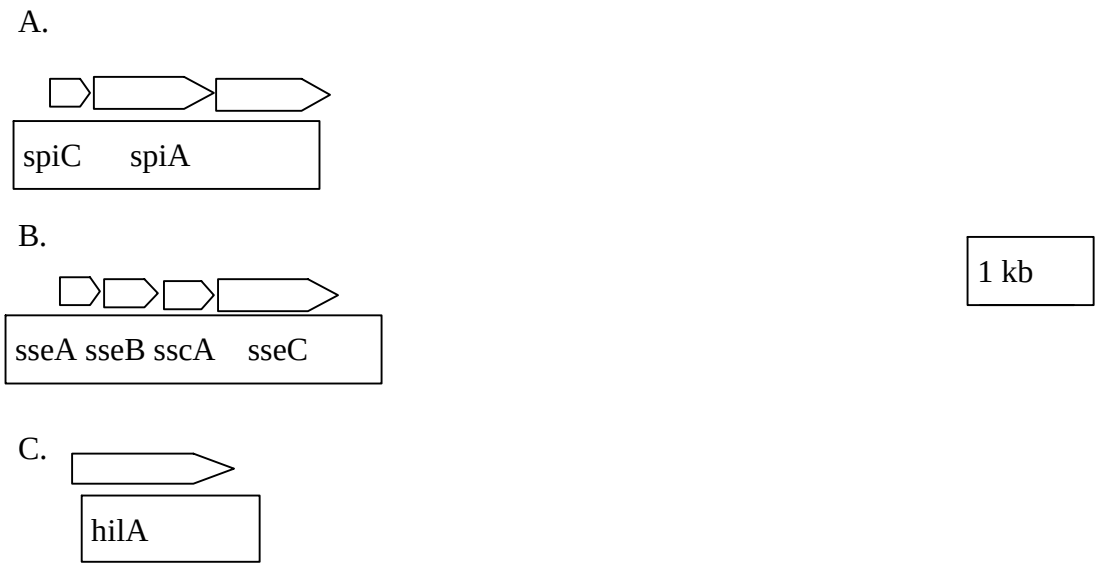

Figure 2: Two fragments contain genes *spiCAB* (A) and *sseAB-sscAC* (B) and one transcriptional regulatory gene (*hilA*) of SPI-1(C)