P003

Characterization of the Genes of *Salmonella typhimurium* conferring the penetration of cultured HEp-2 and Chinese hamster ovary cells

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The invasion genes from *Salmonella typhimurium* were identified by the construction of a cosmid library and subcloning genes into a plasmid vector, pGEM-7Z. The 4.65 kb fragment of the invasion-conferring genomic region of the subclone, pSV6235 was sequenced in both direction. The three open reading frames, which were located at downstream of a promoter region, were designated as *sir* (Salmonella invasion region)*A* coding for the 36 amino acids, *sirB* coding for the 132 amino acids and *sirC* for the 82 amino acids, respectively. Interesingly, the genomic region of pSV6235 was highly homologous to *Yersinia enterocolitica* genomic DNA for a high pathogenicity island and *Salmonella enteritidis* insertion element IS*1351* and IS*200* DNA. These results show that there could be a significant relationship between *S. typhimurium*, *Y. enterocolitica* and *S. enteritidis* with respect to horizontal evolution process and acquisition of virulence determinants by means of transposon, plasmid or bacteriophage.

Introduction

The process of salmonellosis consists of [1)]bacterial attachment on and penetration to the epithelial cells, [2)]circumvention of the host immune system including phagocytosis and selection of a unique niche, and [3)]proliferation. It is generally accepted that invasiveness for the mammalian cells is an integral aspect of the disease caused by the pathogens[1].

The potential invasion genes were identified from genomic DNA of *S. typhimurium* by the construction of a cosmid library, and followed by subcloning the invasion genes into a plasmid vector, pGEM-7Z[2]. The potential invasion genes were sequenced for characterization.

Materials and Methods

S. typhimurium 82/6915 was used to derive putative invasion genes. A *S. typhimurium* cosmid library was constructed to identify the invasion genes involved in Salmonella infections. A genomic library of *S. typhimurium* was cloned into the cosmid vector, pLA2917 using the Packagene Lambda DNA Packaging Extract (Promega, USA). To define the invasion genes, the genomic region carrying the genes derived from *S. typhimurium* was cleaved with *Bgl*II (Progen, Australia) and subcloned into the *Bam*HI site of pGEM-7Zf(+) by standard methods.

Tissue-culture invasion assay using HeLa and Chinese hamster ovary cells (CSIRO, Australia) was performed to identify the clones carrying the invasion genes, as described before[2]. To characterize the invasion genes, the 4.65 kb invasion-conferring genomic region was sequenced in both direction using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA).

Results and Discussion

The potential invasion genes from *S. typhimurium* were identified by the construction of a cosmid library and subcloning the invasion genes into a plasmid vector, pGEM-7Z (Table 1). The 4.65 kb fragment of the invasion-conferring genomic region showed three open reading frames, which were located at downstream of a promoter region. They were designated as *sir* (**S**almonella **i**nvasion **r**egion)*A* coding for the 36 amino acids, *sirB* coding for the 132 amino acids and *sirC* for the 82 amino acids, respectively. Interesingly, the genomic region of pSV6235 was highly homologous to *Yersinia enterocolitica* genomic DNA for a high pathogenicity island and *Salmonella enteritidis* insertion element IS*1351* and IS*200* DNA (Table 2). These results show that there could be a significant relationship between *S. typhimurium*, *Y. enterocolitica* and *S. enteritidis* with respect to horizontal evolution process and acquisition of virulence determinants by means of transposon, plasmid or bacteriophage.

Reference

1. Braude, A. I., C. E. Davis, and J. Fierer. "Infectious diseases and medical microbiology"(1986), VolII, 2nd ed. W. B. Saunders Company. USA.
2. Park, J. U. "Molecular analysis of the genes mediating *Salmonella* invasion"(1997), FEMS Immunology and Medical Mirobiology. 18, p.113-117.

Table 1. Invasion assay of the *Salmonella* cosmids, pSI511 and pSI623 to HEp-2 and chinese hamster ovary (CHO) epithelial cells

Strains	Invasion efficiency[a]	
	HEp-2	CHO
S. typhimurium	0.417 (100)	0.383 (100)
E. coli DH1	0.001 (0.24)	0.005 (0.26)
pSI511[b]	0.043 (10.31)	0.031 (8.09)
pSI623[b]	0.051 (12.23)	0.035 (9.14)

[a](number of intracellular bacteria × 100) / number of bacteria in inoculum.
[b]pLA2917 carrying the 29 kb fragment of *S. typhimurium* genomic region conveying the invasion determinant genes.
[c]pLA2917 carrying the 27 kb fragment of *S. typhimurium* genomic region conveying the invasion determinant genes.
The values shown are the averages of at least three independent experiments conducted in duplication. The bracket values indicate relative percent internalization efficiency, internalization efficiency relative to *S. typhimurium*, representing 100%.

Table 2. DNA homology search data of the 4650 bp invasion-conferring genomic region of pSV6235

Definition	Score
Salmonella enteritidis insertion element	946
Yersinia enterocolitica DNA for a high pathogenicity island	660
Yersinia enterocolitica plasmid DNA fragment	660
E. coli grx gene encoding glutaredoxin	440
E. coli modulator of drug activity (*mda*18) and glutaredoxin	440
E. coli grxA, *ybjc*, *mdaA*, *rimK*, *potF*, *potG*, *potH* and *potI* genes	440
E. coli genomic DNA	440
Yersinia enterocolitica yopA and *ylpA* genes	270
S. typhimurium insert regulator	262
Haemophilus influenzae DNA	223
E. coli cooC and *cooD* genes	216