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Research Progress of Salmonella Pathogenicity Island

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Abstract: *Salmonella* is a zoonotic and food-borne pathogen. The widespread spread and contamination of this pathogen poses a great threat to food safety and public health. The existence of Salmonella virulence island is closely related to its pathogenic effect. In this paper, five main virulence islands of *Salmonella* and their encoding genes and functions are systematically introduced. Further analysis of the characteristics of pathogenicity islands, virulence evolution and virulence gene expression regulation are helpful to explore the mechanism of interaction between various virulence factors and host in *Salmonella*, so as to provide new ideas for the prevention and treatment of salmonellosis.

Keywords: *Salmonella; Salmonella* Pathogenicity Island; Gene Regulation.

1. Introduction

Salmonella is a zoonotic and food-borne pathogen that is widely transmitted worldwide. It can cause gastroenteritis, typhoid fever, septicaemia and other symptoms. Every year, it causes about 93.8 million infections and 155,000 deaths[1]. Based on the difference of 16S rRNA sequence, *Salmonella* can be divided into two species: *Salmonella enterica* and *Salmonella bongori*. Further, *Salmonella enteric* can be divided into six subspecies according to biochemical characteristics and genomic correlation. Almost all cases of *Salmonella* infection in animals are caused by subspecies I. While subspecies II, IIIa, IIIb, IV, VI, and subspecies V (*Salmonella bongori*) are usually isolated from cold-blooded animals and the environment[2]. In addition, *Salmonella enterica* can be divided into more than 60 serogroups and 2600 serotypes based on the difference of somatic antigen (O antigen) and flagellar antigen (H antigen), among which *S.* Typhoid and *S.* Paratyphoid only infect humans and cause severe typhoid symptoms. Serotypes of *Salmonella* such as Enteritis and Typhimurium usually have a wide range of host infections, causing self-limiting diseases such as gastroenteritis [3].

The pathogenicity of *Salmonella* is closely related to its virulence genes[4]. The virulence genes, which typically reside in tight clusters on chromosomes, plasmids or specific areas of bacteriophages, are known as *Salmonella* Pathogenicity Island (SPI). Since the SPIs are obtained by horizontal gene transfer, their specific "mobility" characteristics enable the bacterial genome evolution to take a quantum leap in a short period of time, providing a prerequisite for pathogenic bacteria to adapt to a new ecological niche. So far, 24 kinds of SPIs have been found in *Salmonella*, among which SPI-1 ~ SPI-5 exists in all serotypes of *Salmonella enterica*, and the rest virulence islands are distributed in different species and serotypes of *Salmonella enterica*. Various virulence islands encode different virulence factors, such as pili, secretory proteins, toxins, and regulatory factors, helping *Salmonella* to function at different stages of colonization, invasion, and replication in the host environment[2]. In this paper, the molecular structure, coding genes and main functions of five virulence islands of *Salmonella* were reviewed. In addition, the evolution of virulence, the characteristics of virulence islands and the regulation of virulence genes expression of *Salmonella* were also discussed, which provided references for the study of pathogenic mechanism and prevention and control of *Salmonella*.

2. Salmonella Pathogenicity Island

SPI-1

SPI-1 is a 40 kb DNA fragment located at the 63' site of the chromosome and has stable genetic characteristics, which is present in all *Salmonella* species. SPI-1 contains virulence gene clusters such as *inv*, *spa*, *sip*, *prg*, *org*, *sit*, *hil*, s*i*c, *spt*, *spr* and *avr*, among which at least 29 genes are involved in encoding structural proteins, effect-factors and regulators of T3SS-1. Effectors encoded by SPI-1, such as SipABCD and SptP, contribute to bacterial internalization by inducing cytoskeletal rearrangement of small intestinal epithelial cells[5]. The virulence of mice infected by oral and intraperitoneal injection of SPI-1 mutant *Salmonella* was reduced by 15 to 50 times, indicating that the intraperitoneal injection of *Salmonella* evaded the intestinal barrier. Therefore, the virulence gene encoded by SPI-1 is required for the early colonization and invasion stages of *Salmonella* infection. The *sitABCE* operon on the edge of SPI-1 encodes an iron intake system that does not participate in the invasion process but promotes the secretion of T3SS. In addition, several chaperone proteins are encoded in SPI-1, which maintain the integrity and independence of secreted proteins in bacteria by specific binding to their target proteins and mediate their recognition and transport with T3SS-1[4].

SPI-2

SPI-2 is 39.7 kb in length, located at locus 31' of the *Salmonella* chromosome, and contains more than 40 *Salmonella*-specific open reading frames. SPI-2 exists only in *Salmonella enterica* and is absent in *Salmonella bongori*. It can be divided into two parts based on molecular structure analysis. The smaller part (15kb) contains a cluster of genes encoding tetracycline sulfate reductase (*ttrABC*) and its two-component regulatory system (*ttrRS*), enabling *Salmonella* to breathe using tetracycline sulfate as an electron acceptor under anaerobic conditions. Also included are seven open reading frames with unknown functions. Preliminary results showed that these genes had no significant effect on systemic infection in mice[6]. The larger part (25kb) was located between tRNA *valV* and ORF242, and mainly included four operons (*ssa*, *ssr*, *ssc*, *sse*), encoding structural proteins,

regulatory factors, molecular chaperones, and effector proteins related to T3SS-2, respectively. The secretory system T3SS-2 associated with SPI-2 can transport more than 20 effector factors into the host cytoplasm, controlling the survival and replication of *Salmonella* in epithelial cells and phagocytes. At the same time, these effector proteins enable *Salmonella* to evade phagocytosis and play an important virulence role in the systemic infection of the host[2]. Bispham et al. [7] used signal label mutagenesis to investigate the effects of SPI-2-mutated *S.* Dublin on calves, and the results showed that SPI-2 was closely associated with enteritis and systemic salmonellosis.

SPI-3

SPI-3, about 17 kb in size, is located downstream of the *tRNA selC* site at 82' of chromosome and contains 10 ORFs, constituting 6 transcription units. The virulence island contains the *mgtCB* operon, which encodes a magnesium ion transport system that mediates *Salmonella* survival in a low magnesium environment and replication in macrophages[8]. misL gene encodes MisL, an adhesion protein associated with intestinal colonization, which enhances the invasion of enteroepithelial cells by mediating the binding of fibronectin to host extracellular matrix proteins[8]. In addition, *marT* gene on SPI-3 encodes a regulatory factor responsible for regulating the expression of *misL* and biofilm-related genes [9].

SPI-4

The SPI-4 of *Salmonella* is a 27 kb region located on chromosome 92' and contains six virulence genes *siiABCDEF*. The encoding products SiiC, SiiD, and SiiF proteins constitute the Type I secretion system (T1SS). SiiE is a non-pili adhesion protein with a high molecular weight (~600 kDa) transported by T1SS, mediating the colonization and adhesion of epithelial cells. However, SiiA and SiiB are two intima proteins that are not secreted extracellular, and their functions remain to be determined. However, these two proteins are not necessary for SiiE secretion, and mutations in their respective genes do not seem to affect the expression of SiiE or other SPI-4 gene products[4]. Kiss et al.[10] compared the virulence of 6 gene mutants of *sii* gene cluster with the wild-type *Salmonella* strain, and the results showed that the virulence of the wild-type strain was stronger than the 6 *sii* mutants, indicating that they all had certain significance in the pathogenicity of *Salmonella*.

SPI-5

SPI-5, about 7.6 kb in size, is located at the 25' of *Salmonella* chromosome, flanked by *serT* and *copS*/*copR* loci, and contains at least five virulence genes, *pipA*, *pipB*, *pipC*, pipD and *sopB*. SopB, as a secreted protein, is transferred to the host cytoplasm via T3SS-1 to induce intestinal cell fluid secretion and migration of polymorphonuclear leukocytes (PMNS). SopB also exhibits inositol phosphatase activity, which is directly related to host diarrhea. PipC may be a molecular chaperone that affects the stability of SopB secretion[5]. Wood et al.[11] studied the role of *pipA*, *pipB* and *pipD* genes, and the results showed that the mutants in these genes did not affect the growth rate of the strain and the ability to invade HeLa cells. However, both the inflammatory response and humoral secretion rates were reduced in the pip mutants compared to the wild-type strains, suggesting that these genes are directly related to the enteric pathogenicity of *Salmonella*.

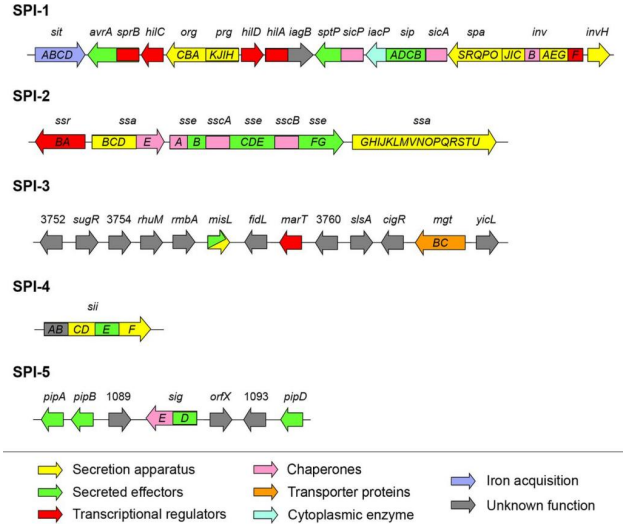


Figure 1. Virulence genes and their functions carried in five SPIs

Table 1. Basic characteristics of five SPIs

SPI	Size (kb)	G+C (%)	Location	Major Functions
SPI-1	39.8	42	*flhA-mutS*	Encoding T3SS-1, involved in the early colonization and invasion of *Salmonella* infections
SPI-2	39.7	44.6	tRNA *valV*	Encoding T3SS-2, related to the survival and replication of *Salmonella* in host cells
SPI-3	34	47.3	tRNA *selC*	It mediates the survival and replication of *Salmonella* in macrophages in a low magnesium environment
SPI-4	27	44.8	tRNA-like	Encoding T1SS, it mediates the colonization and adhesion of thalli and epithelial cells
SPI-5	7.6	43.6	tRNA *serT*	Stimulate signal transduction of pro-inflammatory cytokines

3. Evolution of Salmonella Virulence Island and its Virulence

Evolution is the basic meaning of biological existence, and adaptive selection determines the speed and direction of evolution. All organisms, including pathogenic bacteria, drive their own evolution by adapting to a specific ecological niche. Based on the viewpoint of systems biology, the acquisition of virulence islands can give pathogenic bacteria adaptive advantages and enhance the ability of bacteria to colonize, invade, reproduce and spread in a specific environment. Studies on the various virulence islands of *Salmonella* have found that genes encoding certain virulence characteristics are generally distributed in clusters in a single operon. The "selfish operon" hypothesis holds that the existence of operons increases the probability of horizontal transfer of genes together, and at the same time minimizes the demand of gene expression on ribosome, RNA polymerase, transcription factors and other gene expression machines, thus speeding up the process of gene expression[12][13]. The existence of virulence islands with gene cluster structure enables bacteria to acquire complex pathogenic functions in a single horizontal gene transfer event, which is an effective way to improve the adaptability of pathogenic bacteria. At the same time, it is helpful for pathogenic bacteria to retain the

beneficial gene cluster and spread to the offspring quickly, which affects the process and direction of bacterial evolution to some extent.

The evolution process of pathogen genome is the same as that of other organisms, mainly through point mutation, gene recombination and gene horizontal transfer to achieve modification of existing genes, preservation of beneficial genes and deletion of harmful or useless genes. The way of point mutation is relatively common but the process is slow, while many new varieties can be produced in a short time through the horizontal transfer of large segments of the genome like virulence islands, speeding up the evolution of new pathogens. Early studies confirmed the close relationship between *Salmonella* and E. coli and divided the evolution of *Salmonella* virulence into three stages. In the first stage, Escherichia coli obtained SPI-1 through horizontal gene transfer mediated by phages or plasmids, became *Salmonella*, and had the ability to infect intestinal epithelial cells. In the second stage, two lineages of *Salmonella enterica* and *Salmonella bongori* were evolved through point mutation and other mechanisms. After that, *Salmonella enterica* obtained SPI-2 through horizontal transfer, which endowed *Salmonella* with the ability to survive and replicate in host cells. In the third stage, *Salmonella enterica* further differentiated into six subspecies in the process of improving adaptation to warm-blooded vertebrate hosts.

With the progress of biotechnology and the deepening of research on *Salmonella*, various types of virulence islands have been found in different species and serotypes of *Salmonella*. SPI can be divided into four categories based on its distribution in different species and serotypes of *Salmonella*. The first type is the SPI shared by *Salmonella enterica* and *Salmonella bongori*, which may have been acquired before the evolution of *Salmonella*, such as SPI-1 and SPI-9; The second category is present in all *Salmonella enteric* but absent in *Salmonella bongori*, such as SPI-2; The third type SPI, found in some serotypes of *Salmonella enterica* but missing in *Salmonella bongori*, were probably acquired after the formation of *Salmonella* genus and in the early stages of diversification of *Salmonella enterica*, such as SPI-18; The fourth category is an unstable SPI found only in a small subset of serotype strains, likely the product of recent horizontal gene transfer events, such as SPI-7. It can be seen that the acquisition of SPI accompanied the whole evolutionary process of *Salmonella* and endowed *Salmonella* with adaptability in different environments [14].

4. Characteristics of Salmonella Virulence Island

Hacker et al. [15] systematically studied and explained virulence island for the first time in 1997, and defined it as a gene fragment encoding virulence related genes with relatively high molecular weight, which was constantly modified and improved in subsequent studies. Currently, SPI has the following characteristics.

Encodes virulence related factors and regulatory systems. According to the functional differences of the gene products encoded by virulence islands, they can be divided into the following categories: (1) Adhesion factors, such as pili and adhesins, help pathogens to adhere to the surface of host intestinal cells and promote their colonization; (2) Exotoxins: such as enterotoxins, hemolysin, etc., can interfere with the normal signaling process of host cells, resulting in excessive secretion of body fluids; (3) Secretory system: it can transport the effector proteins of *Salmonella* to neighboring cells or host cells and regulate their functions; (4) Virulence regulatory system: It can regulate the expression of virulence island genes and is controlled by the overall regulatory system, thus giving host bacteria new virulence characteristics in coordination and cooperation.

The G + C content of most SPI was significantly different from that of other locations of bacterial chromosomes. Because the codon preference of exogenous genes may be different from that of host bacteria, the horizontal gene transfer integration into the host chromosome will show obvious differences in G + C content. According to this difference, SPIs can be divided into high G + C pathogenicity islands and low G + C pathogenicity islands. However, due to long-term evolution, the base composition and codon preference of many pathogenicity islands have tended to be consistent with that of *Salmonella* chromosomes, so the difference of G + C content is not obvious.

SPIs are often located near tRNA sites in *Salmonella* chromosomes. tRNA gene is a highly conserved fragment in different species of bacteria, and its symmetrical structure provides suitable action sites for recombinases, becoming an integration hotspot for homologous recombination of foreign DNA and host chromosomes. According to statistics, about 75% of virulence islands are closely related to tRNA genes.

5. Regulation of SPI Gene Expression in Salmonella

In order to ensure that the genes on the pathogenicity island can be expressed at the right time and location, the regulation of gene expression plays an extremely important role in the pathogenic process of *Salmonella*. This regulation is carried out at a time level, and the toxic genes need to be expressed gradually[4].

It has been proved that the expression of various genes on SPI is stimulated by environmental signals of different ecological niches (oxygen content, osmotic pressure, ion concentration, pH, etc.) of the host, and after the SPI was obtained by horizontal gene transfer in the early host bacteria, gene expression can be achieved better only after compatibility with the existing regulatory network of the bacteria. The extremely complex regulation of the SPI gene therefore depends on a balance of interactions between the intracoland and off-island regulators. At present, a variety of regulatory factors have been found to regulate the expression of SPI in *Salmonella*, among which the most important ones include the regulatory factors HilA, HilC, HilD, InvF encoded by SPI-1, the two-component regulatory system SsrA-SsrB encoded by SPI-2, and the global regulatory factors encoded by genes other than SPI. Such as two-component regulatory system PhoQ-PhoP, etc. These regulatory factors interact with each other to form the regulatory network of SPI gene expression.

HilA, a transcriptional activator belonging to the OmpR/ToxR family, can activate a variety of structural proteins and secretory factors encoded by SPI-1[16]. This regulator plays a central role in the invasion of *Salmonella*. Not only do all regulatory systems and environmental signals affect its expression, but both in vitro and in vivo experiments have shown that *hilA* gene mutants significantly reduce the invasion performance of *Salmonell*a[17]. At the same time, HilA can also regulate the expression of *sii* operon of SPI-4

and *sigD* gene in SPI-5 by directly binding with the promoter. However, HilA can inhibit the expression of SPI-2 genes (such as *ssaH* and *sseL*) under invasion-induced conditions [18].

The regulator InvF, a member of the AraC family, is encoded by the first gene of the inv operon in SPI-1 and can be activated in a HILa-dependent or independent manner. InvF works with the chaperone protein SicA to activate the expression of downstream virulence genes. These virulence genes are mainly *sic/sip* operon in SPI-1 and effect-factor coding genes encoded at other positions on the chromosome, such as sptP[19]. In addition, HilD and HilC are also the regulatory factors encoded by SPI-1, which combine with the off-island regulatory factor RtsA. The combined action of the three activators can amplify the signal and jointly control the expression of HilA[20]. In addition, HilD and HilC can activate InvF expression in a HILa-independent way[21].

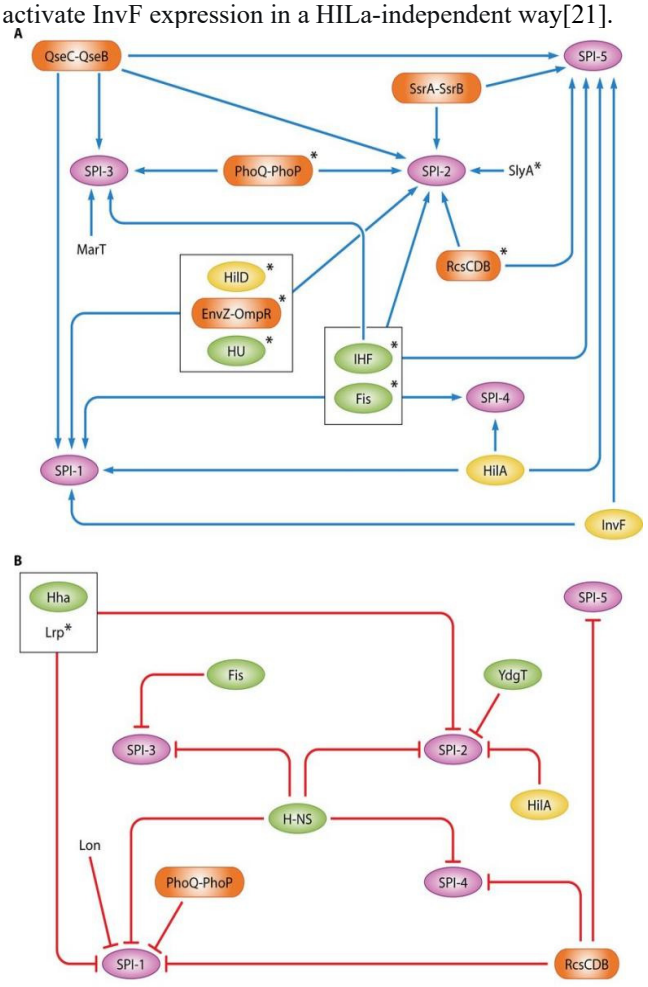


Figure 2. Correlation among SPI and gene regulation mechanism [4]. (A) Regulatory factors positively affected SPI gene expression. (B) The regulator that causes the inhibitory effect. The regulator that acts on the SPI-2 gene in this model via SsrA-SsrB is marked with an asterisk.

The SsrA-SsrB two-component regulatory system is the most important and basic regulatory system for gene expression in SPI-2. SsrA is an induction protein located on the membrane, and SsrB is a transcription regulatory factor. SsrA senses the corresponding molecular signals and transmits them to SsrB through phosphorylation, thus regulating the expression of downstream genes[22]. Interestingly, there are two promoters in the *ssrAB* operon, one upstream of each gene, leading to different regulation and uncoupling production, and there is evidence that SsrB can automatically regulate and activate SsrA expression[23].

In addition to the regulators encoded by SPI, several global regulators regulate the expression of virulence islands. For example, the two-component regulatory system PhoQ-PhoP. Low bivalent cations (Mg^{2+} and Ca^{2+}) concentrations and low pH activate the induction protein PhoQ, which in turn activates the expression of the regulatory factor PhoP. PhoP promotes bacterial survival in host macrophages by inhibiting or activating the expression of some genes within SPI-1 and SPI-2. In addition, PhoP also regulates *mgtCB* in SPI-3 and *sii* operon in SPI-4. BarA-SirA is also an integral two-component regulatory system, which is involved in carbohydrate metabolism, biofilm formation, locomotion and host invasion in bacteria[24]-[25].

6. Conclusion

SPI codes most of the virulence factors of *Salmonella*, and various virulence islands play a role in the pathogenic process of bacteria by expressing their respective functional proteins. In this paper, we elucidate the structure, encoding genes and functional information of five virulence islands of *Salmonella*, and discuss the regulation system related to gene expression. Although a variety of virulence islands have been discovered, the complex coordination mechanism of individual virulence islands in the pathogenesis remains to be further studied. An in-depth study of virulence Island will not only help us to understand the complex microbial world and the mechanism of the emergence of new pathogenic microorganisms, but also provide a reliable basis for the prevention and control of infectious diseases.

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