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• ORIGINAL RESEARCH •

Relationship between genotype and phenotype of flagellin C in Salmonella

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Abstract

AIM: **To discover the relationship between the genotype and antigen serotype of flagellin C among Salmonella strains.**

METHODS: **Fragment of Salmonella flagellin C in plasmid pLS408 was cloned, sequenced and compared with the corresponding sequence in other strains. Salmonella strains including two typhi strains, one paratyphoid strain, one enteritidis and one typhimurium strain were isolated from outpatients. Genome DNA was purified respectively from these clinical isolates, then the corresponding flagellin C fragment was amplified by polymerase chain reaction, and the amplification products were analyzed by agarose gel electrophoresis.**

RESULTS: **The cloned fragment includes 582 nucleotides encoding the variable region and partial conservative region of Salmonella flagellin C in plasmid pLS408. With comparison to the corresponding sequences reported previously, there is only a little difference from other strains with the same flagellar serotype in both nucleotide and amino acid level. Specific PCR products were amplified in Salmonella strains with flagellar serotype H-1-d including *S. muenchen*, typhi and typhimurium, but not in *S. paratyphoid* C or S. enteritidis strains.**

CONCLUSION: **In this experiment, the specificity of nucleotide sequence could be found in flagellin C central variable regions as it exists in flagellar serotypes in Salmonella. It may be helpful to developing a rapid, sensitive, accurate and PCR-based method to detect Salmonella strains with serotype H-1-d.**

Subject headings: Salmonella; flagellin C; polymerase chain reaction; serotype; genotype

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INTRODUCTION

Bacteria of genus Salmonella are both major and minor pathogens that cause disease outbreaks that arise from single incidents of breakdown in food hygiene. Much effort has been devoted to methods that differentiate these organisms in order that a given outbreak may be traced to its source and breakdown. Serological analysis of the surface antigens, such as flagellar antigen, has been proven to be the most suitable approach and has resulted in the recognition of a large number of serotypes.

In the past decade, the nucleotide sequences of flagellin in several Salmonella strains were published, the information of secondary and tertiary structure of flagella was discovered by chemical methods and X-ray crystallogram, and the antigen variable region was also determined roughly. These results indicate that serotype of flagella is determined by the linear primary structure of flagellin and the strain with different serotypes has different amino acid sequence in the central region of flagellin.

According to the standpoint that phenotype is determined by corresponding genotype, it is reasonable to think that the differentiation in the variable region of flagellin may be reflected by the nucleotide sequence. Detection of nucleotide difference by specific amplification may be helpful for the diagnosis and typing of Salmonella.

MATERIAL AND METHODS

Strains and plasmid

Salmonella 5930, which contains plasmid pLS408, was offered by Professor Stocker (Stanford University School of Medicine). Plasmid pUC18 and Eschrichiacoli. DH-5á was stored in our laboratory. Salmonella strains including two typhi strains, one paratyphoid strain, one enteritidis and one typhimurium strain were isolated from outpatients, and samples were stored in Department of Bacteriology, Xijing Hospital.

Cloning of partial flagellin C gene fragment

Plasmid pLS408, in serted with the complete sequence of flagellin C from *S. muenchen*[1], was prepa red as template by plasmid purification kit (Shanghai Watson Biotech. LTD). Primers, including EcoRV and *KpnI* sites for convenient cloning, were designed referring to the complete sequence of flagellin C in *S. muenchen* and *S. typhi*. Forward primer: 5' GCAGGATATCTTCCTCGAGACCACAGTTGCGGCTC 3'; reverse primer: 5' TGCGCC AGAACGGAGGTACC 3'. PCR product was digested by EcoRV and *KpnI*, ligated to pUC18 digested by *HinCII* and *KpnI* and sequenced in Sangon.

Amplification of flagellar serotype specific fragment in Salmonella strains

Genome DNA was prepared by genome purification kit according to the Watson's Handbook for DNA Isolation an d Purification. Amplification was performed by two-step polymerase chain reaction, i.e. preheating at 94℃ for 5 min, then denaturing at 94℃ for 45 s and an nealing at 68℃ for 1 min, 30 cycles with a final extension at 72℃ for 10 min. Amplification products were analyzed by 1.5 $g \cdot L^{-1}$ agarose gel electrophoresis.

RESULTS

The map of plasmid pLS408 and the result of agarose gel electrophoresis of fliC PCR product were demonstrated (Figure 1).

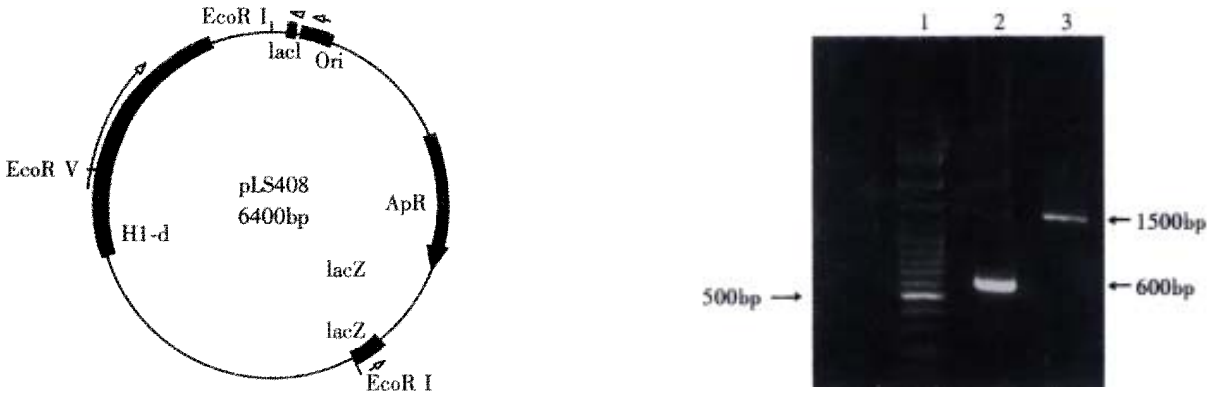

Figure 1 Map of pLS408 (left) and gel electrophoresis of PCR p roduct (right).
Lane 1: 100 bp DNA ladder, lane 2: PCR product of partial flagellin C gene, lane 3: PCR product of flagellin C gene.

The amplification product includes 582 nucleotides encoding variable region and partial conservative region of flagellin C. In comparison with the corresponding sequences reported before, there is only a little difference from other strains with the same flagellar serotype in both nucleotide and amino acid level (Figures 2 and 3). Compared with the known *S. muenchen* sequence, there are three single-nucleotide insertions, which cause frame shift and ten amino acid changes (166-195). T here are three single-nucleotide mutations, which cause two missenses (23:C to T, 136:G to A) and one silent mutation (216: G to T), and a double-nucleotide mutation (281, 282:TA to AT) and a three-nucleotide short fragment mutation (217, 218, 219: CTC to GCT), which causes one missense mutation, respectively. Though t here are some differences between the sequence here and that derived from the other *S. muenchen* strain, it is conservative highly with the corresponding sequence in Salmonella strains with the same flagellar serotype.

1	ACCACAGTTGCGGCTCAACTTGTTGCTGCAGGGGTTACTGGTGCCGATAAGGACAATACT
	ACCACAGTTGCGGCTCAACTTGCTGCTGCAGGGGTTACTGGTGCCGATAAGGACAATACT
61	AGCCTTGTAAAACTATCGTTTGAGGATAAAAACGGTAAGGTTATTGATGGTGGCTATGCA
	AGCCTTGTAAAACTATCGTTTGAGGATAAAAACGGTAAGGTTATTGATGGTGGCTATGCA
121	GTGAAAATGGGCGACAATTTCTATGCCGCTACATATGATGAGAAAACAGGTACAATTACT
	GTGAAAATGGGCGACGATTTCTATGCCGCTACATATGATGAGAAA_CAGGTACAATTACT
181	GCTAAAACAACCACTTATACAGATGGTGCTGGCGTTGCTCAAACTGGAGCTGTGAAATTT
	GCTAAA_CAACCAC_TATACAGATGGTGCTGGCGTGCTCCAAACTGGAGCTGTGAAATTT
241	GGTGGCGCAAATGGTAAATCTGAAGTTGTTACTGCTACCGATGGTAAAACTTACTTAGCA
	GGTGGCGCAAATGGTAAATCTGAAGTTGTTACTGCTACCGTAGGTAAAACTTACTTAGCA
301	AGCGACCTTGACAAACATAACTTCAGAACAGGCGGTGAGCTTAAAGAGGTTAATACAGAT
	AGCGACCTTGACAAACATAACTTCAGAACAGGCGGTGAGCTTAAAGAGGTTAATACAGAT
361	AAGACTGAAAACCCACTGCAGAAAATTGATGCTGCCTTGGCACAGGTTGATACACTTCGT
	AAGACTGAAAACCCACTGCAGAAAATTGATGCTGCCTTGGCACAGGTTGATACACTTCGT
421	TCTGACCTGGGTGCGGTACAGAACCGTTTCAACTCCGCTATCACCAACCTGGGCAATACC
	TCTGACCTGGGTGCGGTACAGAACCGTTTCAACTCCGCTATCACCAACCTGGGCAATACC
481	GTAAATAACCTGTCTTCTGCCCGTAGCCGTATCGAAGATTCCGACTACGCGACCGAAGTC
	GTAAATAACCTGTCTTCTGCCCGTAGCCGTATCGAAGATTCCGACTACGCGACCGAAGTC
541	TCCAACATGTCTCGCGCGCAGATTCTGCAGCAGGCCGGTACC*
	TCCAACATGTCTCGCGCGCAGATTCTGCAGCAGGCCGGTACC#

Figure 2 Comparison of nucleotide sequence between PCR product and the corresponding sequence reported previously (all differences are shadowed or dashed).
*Sequence of partial flagellin C PCR product, #Sequence from Genbank X0 3395

1	TTVAAQLVAAGVTGADKDNTSLVKLSFEDKNGKVIDGGYAVKMGDNFYAATYDEKTGTITAKTTT
	TTVAAQLAAAGVTGADKDNTSLVKLSFEDKNGKVIDGGYAVKMGDDFYAATYDEKQVQLLLNN
	TTVAAQLAAAGVTGADKDNTSLVKLSFEDKNGKVIDGGYAVKMGDDFYAATYDEKTGAITAKTTT
66	YTDGAGVAQTGAVKFGGANGKSEVVTATDGKTYLASDLDKHNFRTGGELKEVNTDKTENPLQKID
	YTDGAGVLQTGAVKFGGANGKSEVVTATVGKTYLASDLDKHNFRTGGELKEVNTDKTENPLQKID
	YTDGTGVAQTGAVKFGGANGKSEVVTATDGKTYLASDLDKHNFRTGGELKEVNTDKTENPLQKID
131	ALAQVDTLRSDLGAVQNRFNSAITNLGNTVNNLSSARSRIEDSDYATEVSNMSRAQILQQAGTS*
	AALAQVDTLRSDLGAVQNRFNSAITNLGNTVNNLSSARSRIEDSDYATEVSNMSRAQILQQAGTS#
	AALAQVDTLRSDLGAVQNRFNSAITNLGNTVNNLSSARSRIEDSDYATEVSNMSRAQILQQAGTS&

Figure 3 Comparison of amino acid sequences among different strains with (all differences are shadowed).
*Amino acid sequence of PCR product. #The corresponding sequences of *S. muenchen* (Genbank X03395). &The corresponding sequence of *S. typhi* (Genbank L21912)

By polymerase chain reaction, amplification products appeared only in those salmonella strains with specific flagella antigen type (H-1-d) as *S. muenchen*, typhi or typhimurium, but not in *S. paratyphoid* C or *S. enteritidis* (Figure 4).

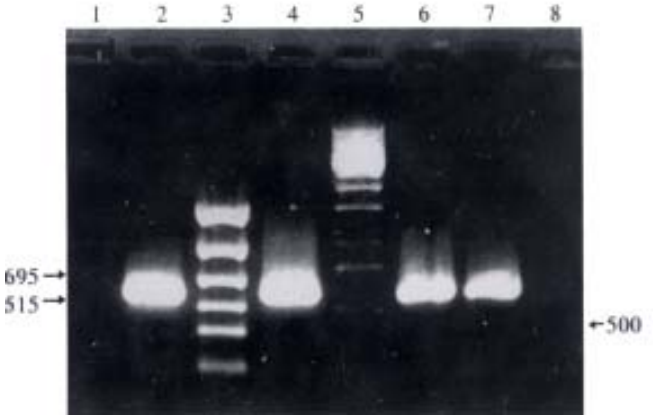

Figure 4 Amplification of flagellin C gene fragment in different Salmonella strains.
Lane 1: S. enteritis; 2,4: *S. typhi*; 3: PCR DNA marker; 5: 100 bp DNA lad der; 6: *S. typhi*murium; 7: *S. muenchen* (plasmid pLS408); 8: S. typhoid.

DISCUSSION

Flagella is a necessary organelle for bacterial motility and its biological behavior is well controlled[2-23]. The flagellar filament is composed of a single protein, flagellin. Serological analysis has proved that diverse types exist among different Salmonella strains[24-26]. The a minoacid sequence of flagellin has been assigned to four domains of flagellin subunit structurally identified in the filament structure, based on biochemical, immunological and structural information[27,32]. The terminal regions form the c ore of t he filament and the central regions form the outer part. Despite the high conservatism in terminal regions, the great divergence in central regions was discovered in both amino acid and nucleotide sequence among the different Salmonella strains. This point was also strongly supported by our experiments.

By comparison with other sequences in Genbank, we found the conservatism of flagellin C central regions among the different strains with the same flagellar antigen type. It is the foundation for the design of primers. In this experiment, primers were designed by software (primer 3 on the Internet) to amplify the flagellin C gene with specific antigen type H-1-d. The sequence of flagellin C variable region in *S. muenchen* was sequenced and compared with other sequence. The cloned fragment was highly aligned with other strains. In fact, the specific amplification occurred in all isolates with H-1-d serotype like *S. muenchen*, typhi or typhimurium, but not in other serotypes like *S. enteritidis* or typhoid C strain and the negative amplification was found in other bacteria like *Helicobacter pylori* is olates or *Eschrichia Coli.* strains (data not shown).

As a developed tool, PCR-bas ed technique has been widely used in both basic and clinical research[33-58]. In bacterial taxonomy, PCR-based genotype typing is complementary to serological typing[59-61]. Furthermore, as a rapid and convenient method, polymerase chain reaction is very helpful to the detection of many microbes like *H. pylori*, Salmonella, crypt ococcal neoformans and many other microbes [62-66]. In this experiment, the specificity of nucleotide sequence could be found in central variable regions of flagellin C as it exists in flagellar serotypes in Salmonella.

In summary, the specificity of nucleotide sequence was found in central variable regions of flagellin C as it exists in flagellar serotypes in Salmonella in this experiment. It may be helpful in the develop ment of a rapid, sensitive and accurate method to detect Salmonella strains with serotype H-1-d.

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