

## The Cloning and Sequencing Analysis of Peptidylprolyl *cis* transisomerases C (*ppiC*) gene of *Salmonella* Typhimurium

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*Salmonella enterica* subspecies *enterica* serovar Enteritidis (SE) and *Salmonella enterica* subspecies *enterica* serovar Typhimurium (ST) are the zoonotic *Salmonella* that colonizes in the intestine of poultry asymptotically and are transmitted to human by consumption of contaminated foods like meat, eggs and egg products. The PPIases are enzymes that accelerate folding of proline-containing proteins by catalyzing the *cis-trans* isomerisation, which plays important roles in a variety of stress responses in bacterium. In this study, the Peptidyl prolyl *cis-trans* isomerases C (*ppiC*) gene from ST was cloned, sequenced, analyzed and submitted to gene bank (accession number KR054755). The retrieved sequence data analysis, suggested that gene belongs to the parvulin family of peptidyl-prolyl *cis/trans* isomerases (PPIases, EC 5.2.1.8) of ST. Entertainingly, the *ppiC* sequence shows about 100% -99% with other *Salmonella* serovars. The conclusion of this study effectively indicate that *ppiC* gene of *Salmonella* is highly conserved in most of the pathogenic *Salmonella* spp. as analogous to patterns of other serovars.

**Keywords:** *Salmonella*, parvulin, PPIases C and *ppiC*.

*Salmonella* are well known for their impact on human and animal health as they are associated with a wide range of clinical problems, including typhoidal and non-typhoidal infections. Non-typhoidal *Salmonella* infections, mainly resulting from *S. Enteritidis* and *S. Typhimurium*, are commonly the result of the consumption of poultry derived food, mainly meat, eggs and egg products (Schroeder *et al.*, 2005, Zaidi *et al.*, 2006, Stephens *et al.*, 2008). *Salmonella* species are responsible for a variety of acute and chronic diseases in poultry. Avian salmonellosis

can develop as a result of infection with poultry-specific serovars, *S. Gallinarum* and *S. Pullorum*, causing systemic illness in birds as well as other *Salmonella* serotypes, including *S. Typhimurium* and *S. Enteritidis* and many others, which contribute to paratyphoid infections (Gast, 2007). The global burden of non-typhoidal salmonellosis was estimated to be about 93.8 million cases per year and of this 80.3 million are food borne (Majowicz *et al.*, 2010) which means more than 80 % of cases of *Salmonella* infection are foodborne. Various serotypes of *Salmonella* are implicating the food-borne infections, but the most prevalent serotypes isolated from humans are *S. Typhimurium* and *S. Enteritidis* (Grahamet *et al.*, 2000). In India some reports showed the predominance of *S. Enteritidis* during 1994-1996 (Verma *et al.*, 2001) but later

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*S. Typhimurium* was found to be the predominant species (Singh, 2002).

Being intracellular the bacterium undergoes various stress conditions generated by the host. Various genes come in to play during stress conditions in the *Salmonella*. The Peptidylprolyl*cis*–*trans* isomerases are the chaperons encoded by various genes found to be involved in the mitigation of stress response. The molecular chaperones are the important proteins, facilitates correct folding and maturation of proteins in Gram-negative bacteria. Peptidylprolyl*cis*/*trans*isomerases (PPIases) catalyze the isomerization of prolyl peptide bonds. The four families of this class of enzymes are cyclophilins (Cyp), FK506-binding proteins (FKBPs), and pavilions which involved in protein folding (Lu et al., 2007). The Peptidylprolyl*cis*–*trans* isomerases are ubiquitous proteins and are found in both prokaryotic and eukaryotic cells (Chouet et al., 1997). The first member of the parvulin subfamily was identified in *E. coli* in 1994 and consists of only 92 amino acids, thus named parvulin (Rahfeld et al., 1994), whose gene was later designated *ppiC*. The PPIases are encoded by many different genes *fkpA*, *fkpB*, *slyD*, *cypD*, *slpA*, *ppiA*, *ppiB*, *ppiC* and *surA* (Ramseier et al., 2013). Parvulins constitute an important PPIase subfamily. *SurA* was the only known member of the parvulin class of PPIases in the periplasm of *E. coli*, however in ST *ppiC* and *ppiD* also member of parvulin class. Dartigalongue and Andrain (1998) reported that in *E. coli* *ppiD* is that its gene is regulated by both the Cpx two-component system and the  $\sigma^{32}$  heat shock factor, known to regulate the expression of cytoplasmic chaperones.

Parvulin proteins have also been reported earlier from *Arabidopsis thaliana* (Landrieu et al., 2000), *Digitalis lanata* (Metzner et al., 2001) and *Malus domestica* (Yao et al., 2001) and the activity is modulated by stress conditions, thus suggesting that it may also be involved in stress adaptation. *surA* gene mutant ST was protected against virulent challenge with the parental strain and to deliver a heterologous antigen to the murine immune system (Sydenham et al., 2000). Humphreys et al. (2003) showed that *SurA* and to a lesser extent *fkpA*, are important for the survival of *S. Typhimurium* in the mouse. However, the studies on the *ppiC* gene of ST hardly noticed. The current study was conducted to

clone the *ppiC* gene of *Salmonella Typhimurium* in pET28C vector followed by sequencing and analyzing the gene with different virulent serovars of *Salmonella*.

## MATERIALS AND METHODS

### Bacterial strain

Poultry isolate *Salmonella Typhimurium* (ST) strain 3232 was procured from the Microbial Type Culture Collection and Gene Bank (MTCC) Chandigarh, India was used in the study. The culture was revived by growing in LB broth at 37 °C overnight and the isolated colonies were obtained by streaking on Hektoen Enteric Agar (HEA) plates. The isolated colonies were confirmed through purity, morphology and biochemical characteristics as per standard protocol. In addition, ST is also further performed by serotyping at National *Salmonella* Centre, Indian Veterinary Research Institute, Izatnagar, India.

### Genomic DNA isolation

The single isolate colony of *Salmonella Typhimurium* inoculate in LB broth and grown at 37 °C, 180 rpm overnight. The overnight grown culture was pelleted (3 ml) for genomic DNA isolation. The DNA was isolated by pure link genomic DNA isolation kit following the manufacturer's protocol (Invitrogen, USA). The concentration and quality of the genomic DNA were assessed by UV absorbance and electrophoresis on 1% agarose gel.

### PCR amplification of *ppiC* gene from *Salmonella Typhimurium*

The primers were designed by using Oligo Analyzer software targeting the PPIases C (*ppiC*) (STM3910) DNA sequence (NCBI-Gene ID: 1255436 Region: 4118944 - 4119225). PCR reaction was performed in a total volume of 100  $\mu$ l containing 50 ng of DNA, 10 pmol of each primer (Table 1), 200  $\mu$ M dNTP, 10  $\mu$ l of 10X PCR buffer, 1.5 mM MgCl<sub>2</sub> and 2.5 U of *Taq* DNA polymerase. The final volume was made with nuclease-free water. The PCR cycling conditions consists of initial denaturation at 95°C for 5 min, followed by 30 cycles each of denaturation (95°C for 30 s), annealing (52 °C for 30 s) and extension (72°C for 30 s) and it was ended with a final extension at (72°C for 10 min.). Finally, the amplified DNA of *ppiC* gene was visualized by electrophoresis on 1% agarose gel and *ppiC* a gene fragments

were purified from gel purified using QIA quick gel extraction kit (Qiagen, Germany) using manufacturers protocol.

The purified PCR product was digested with restriction endonuclease enzyme *NheI* for 5 h at 37°C followed *BamHI* for 1 h at 37°C. Finally, the enzymes were inactivated by incubating at 80°C for 10 min. The RE digested *ppiC* PCR product was again gel purified. In the similar manner the pET28c vector was also digested with *NheI* and *BamHI* restriction enzymes to generate complementary overhangs regions. The RE digested vector was then dephosphorylated at 37 °C for 30 min using shrimp alkaline phosphatase enzyme followed by heat inactivation at 80 °C for 10 min. The digested and dephosphorylated pET28c was gel purified.

#### Cloning of *ppiC* gene

The ligation of the gel purified *ppiC* gene in pET28c expression vector achieved by mixing them in 3:1 ratio followed by incubation at room temperature for 1 h and then at 4°C for 16 h. The ligation reaction was transformed to *E. coli* DH5 $\alpha$  strain competent cells and dispensed on agar plate containing 30  $\mu$ g/ mL of kanamycin. The plates were incubated at 37 °C for overnight. A number of recombinant colonies were assayed by colony PCR. After selecting the recombinant clones, the recombinant pET28c\_ *ppiC* plasmid was extracted from overnight grown culture. The recombinant plasmids were digested by *NheI* and *BamHI* enzymes to confirm insert release. The PPIasesC gene cloned in pET28c plasmid was confirmed by sequencing (GCC biotech).

The sequencing results were assembled and analyzed. Further, the nucleotide sequence was subjected to nucleotide BLAST to compare for sequence similarities with other sequences available in the NCBI database. The final sequence was aligned with the available PPIasesC (*ppiC*) gene of *S. enterica* serovar Typhimurium str. LT2. The sequence was submitted to NCBI

Genbank (<http://www.ncbi.nlm.nih.gov/nucleotide/KR054755>).

## RESULTS AND DISCUSSION

Poultry isolate *Salmonella* Typhimurium strain (3232) was used in present study showed characteristic biochemical, morphological and serological characteristics. On HEA plate, the isolate produced smooth, black centered colonies with greenish periphery (Fig. 1). The ST colonies were confirmed through biochemical and serotyping (Phagoet *et al.*, 2015).

The genomic DNA of *S. enterica* serovar Typhimurium extracted using a commercial kit showed a concentration of 40 ng/  $\mu$ L and good quality on agarose gel (data are not shown). The full gene-coding region of the PPIasesC gene of *S. Typhimurium* was amplified using designed specific primers (Table -1). The size of PCR amplified product on agarose gel electrophoresis for PPIasesC (*ppiC*) gene was 282 bp (Fig. 2). The PCR products were cloned into a pET28c expression vector and this resulted in a recombinant pET28c\_ *ppiC* plasmid that was transformed successfully into *E. coli* bacteria. The recombinant plasmids from the colonies were PCR positive against the *ppiC* gene.

The recombinant pET28c\_ *ppiC* plasmids were confirmed by double digestion with *NheI* and *BamHI* restriction enzymes. Release of the 282 bp amplified product from recombinant vectors by RE digestion further confirmed the gene cloning (Fig. 3). The sequence thus obtained was submitted to GenBank, National Centre for Biotechnology information under the accession no. KR054755. Nucleotide sequence analysis showed that the amplified genes possessed the correct reading frame with compared to other *Salmonella* serovar.

The obtained sequence was subjected to nucleotide BLAST search revealed a high

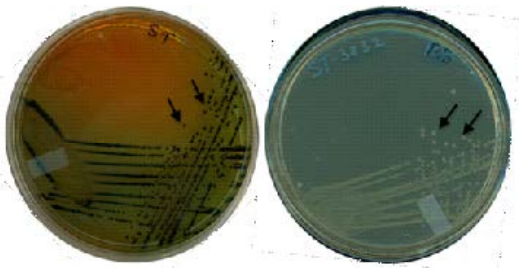
**Table 1.** Primers used in present study

S. No	Primer name	Primer Sequences (5' to 3')	Purpose
1.	PPIase C_For	TATATGCTAGCATGGCAAAAATGGCAGCAG(31)	Amplification of PPIases C( <i>ppiC</i> )gene from ST
2.	PPIase C_Rev	TATATGCTAGCTTATTTACGATACAATACCTTA(34)	

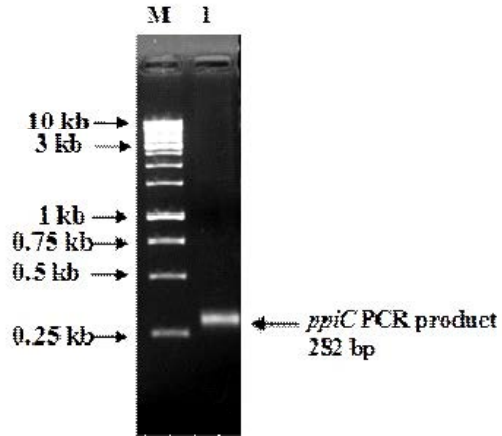
degree of similarity with other *Salmonella* serovar (Table 2). Maximum identity was obtained with *Salmonella* Typhimurium. The percent of identity with *Salmonella* Typhimurium LT2 *ppiC* gene was 100% (Fig. 4). The obtained sequence showed high similarity with different serovar of *Salmonella* strains like 33676, YU39, CDC2011K-0870, ATCC 13311, CFSAN002064, CFSAN001921, STMD1, CFSAN001588, CT18, and ATCC 9120. The *ppiC* gene is highly conserved in most of the

**Table 2.** Results of nucleotide blast showing comparison of PPIase C gene of *S. enterica* serovar Typhimurium strain 3232 with other *Salmonella* serovar

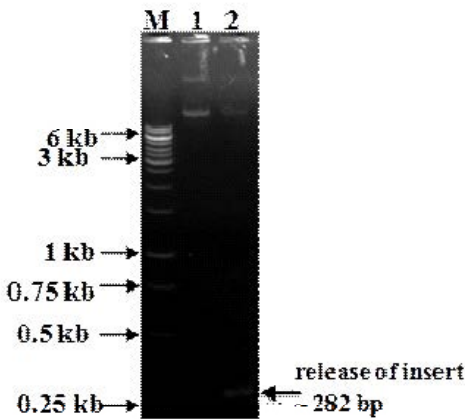
No.	Description	Max score	Total score	Query cover	Ident	Accession
1.	<i>Salmonella enterica</i> subsp. enteric serovar Typhimurium strain 33676, complete genome	521	521	100 %	100 %	CP012681.1
2.	<i>Salmonella enterica</i> subsp. <i>enterica</i> strain YU39, complete genome	521	521	100 %	100 %	CP011428.1
3.	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium str. CDC 2011K-0870, complete genome	521	521	100 %	100 %	CP007523.1
4.	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium strain ATCC 13311, complete genome	521	521	100 %	100 %	CP009102.1
5.	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Heidelberg str. CFSAN002064, complete genome	521	521	100 %	100 %	CP005995.1
6.	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium str. DT2, complete genome	521	521	100 %	100 %	HG326213.1
7.	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium var. 5- str. CFSAN001921, complete genome	521	521	100 %	100 %	CP006048.1
8.	<i>Salmonella typhimurium</i> fragment STMD1	521	521	100 %	100 %	AF233324.1
9.	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Cerro str. CFSAN001588, complete genome	516	516	100 %	99 %	AF233324.1
10.	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Paratyphi A strain CMCC50093 genome	516	516	100 %	99 %	CP011967.1
11.	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhi str. CT18, complete chromosome	516	516	100 %	99 %	AL513382.1
12.	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhi Ty2, complete genome	516	516	100 %	99 %	AE014613.1
13.	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Sloterdijk str. ATCC 15791, complete genome	516	516	100 %	99 %	CP012349.1
14.	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Enteritidis strain OLF-SE9-10012, complete genome	516	516	100 %	99 %	CP009091.1
15.	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Pullorum str. ATCC 9120, complete genome	505	505	100%	99 %	CP012347.1



**Fig. 1.** *S.typhimurium* (3232) growth on HE agar and LB agar plates. *S. Typhimurium* strain 3232 (ST) was sub-cultured and grown in LB-broth media. After that broth culture was streaked on HE agar and LB agar plates. The isolated colonies (indicated by arrow) showed typical morphology of *salmonella*.



**Fig. 2.** PCR amplification of *ppiC* gene. Electrophoresis *ppiC* gene PCR product was performed on 1 % agarose gel. Lane M is 1 kb DNA ladder. Lane 1 is expected *ppiC* PCR product 282 bp (marked by arrow)



**Fig 3:** NheI and BamHI digestion of *ppiA*-pET28c clones. 1 % Agarose gel electrophoresis of RE digested clones. Lane M is 1 kb DNA ladder. Lane 1 is uncut and Lane 2 is cut recombinant plasmids. Released *ppiC* band is shown by arrow.

Salmonella enterica subsp. enterica serovar Typhimurium str. LT2, complete genome  
 Sequence ID: gb(AE006468.1) Length: 4857432 Number of Matches: 1  
 Range 1: 4118944 to 4119225

Score	Expect	Identities	Gaps	Strand	Frame
521 bits(282)	2e-144()	282/282(100%)	0/282(0%)	Plus/Minus	

Features:  
 peptidyl prolyl cis-trans isomerase C

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Query_1      ATGGCAAAAATGGCAGCAGCACTGCATATTCTGTAAAAGAAAGAAACTGGCTTTAGAT
Subject_4118925 ATGGCAAAAATGGCAGCAGCACTGCATATTCTGTAAAAGAAAGAAACTGGCTTTAGAT
Query_61     CTTCTGGAGCAAAATTAAGAACCGCCGCAATTTTGAAGAACTGGGAAAGAAAGCATTCATC
Subject_4119145 CTTCTGGAGCAAAATTAAGAACCGCCGCAATTTTGAAGAACTGGGAAAGAAAGCATTCATC
Query_121    TGGCATGGGTAAAAAAGCGGTGATTTAGGCGAATTTGCTCAGGGGCAAGTGGTTGGG
Subject_4119105 TGGCATGGGTAAAAAAGCGGTGATTTAGGCGAATTTGCTCAGGGGCAAGTGGTTGGG
Query_181    GCATTGGATTAAGTACTGTTTTCCTTGGCCGTACTGGAGCGAAGCGGGCCGCTGGCATTCC
Subject_4119045 GCATTGGATTAAGTACTGTTTTCCTTGGCCGTACTGGAGCGAAGCGGGCCGCTGGCATTCC
Query_241    CAGTTGGTTTACCACTCATTAAGGTATTGTATCCTTAATATAA 282
Subject_4118985 CAGTTGGTTTACCACTCATTAAGGTATTGTATCCTTAATATAA 4118944
    
```

**Fig. 4.** Sequence analysis of peptidyl-prolyl *cis-trans* isomerase C (*ppiC*) from positive clones similar to 100% homology: Query- PPIase C sequence obtained by sequencing, subject-retrieved PPIase C sequence of *S. Typhimurium* LT2 strain from NCBI

pathogenic serovar of *Salmonella* as evidenced from the sequence analysis (Table 2).

*Salmonella* replicates inside the hostile niche of macrophages, and surviving the host depends upon its ability to defend / neutralize phagocyte generated oxidants. Under stress the reactivation of damaged proteins would be energetically favorable than new synthesis. Therefore, bacteria would go for repair the damaged proteins rather than their *de novo*

synthesis (Visick and Clarke 1995).PPIases play a fundamental role in catalyzing the correct folding of many proteins that are non functional in a variety of biological functions and stress (Gething and Sambrook, 1992).

**CONCLUSION**

It may be concluded that ppiases catalyze the peptidyl-prolyl *cis-trans* isomerization, the

rate-limiting step of protein folding, which is essential for newly synthesized protein to get its correct functional form. They can also catalyze the isomerization of that protein which defeat during the oxidative stress and physiological operation (Fischer and Aumuller, 2004). The PpiC enzyme is responsible for the refold of the proteins to save its functions by repairing and also highly conserved in nucleotides sequences among the pathogenic *Salmonella* serovar. The similarity in the sequences of the cloned product with other *Salmonella* serovar suggests its importance in the utility of *ppiC*.

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