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Original Article

Gel Mobility assay of DNA- Pho P/Pho Q Protein Interaction of MDR *Salmonella typhi* isolated from Erode Slaughter House

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Abstract

PhoP and PhoQ is a DNA binding protein in *Salmonella typhi* plays a vital role in expression of virulence. In the present study, Multiple drug resistant (MDR) *Salmonella typhi* isolated from slaughter house and confirmed its antibiotic pattern by Kirby-Bauer principle. The gel documentation of isolated plasmid DNA of antibiotic resistant *S. typhi* along with PhoP/Q protein clearly indicated its interaction. Chemical modification of basic amino acids of PhoP/Q inhibited the DNA protein interaction which was evident through gel mobility shift assay (GMSA). Among the chemical modifiers Di-ethyle pyrocarbonate was effective in inhibiting the above interaction indicating that histidine is the major amino acid involved in such DNA protein interaction. Hence the present study could state that by blocking the basic amino acid histidine's function the virulence of the *S. typhi* can be controlled.

Key words: MDR *Salmonella typhi*, phoP/phoQ Protein, Gel Mobility Shift Assay (GMSA), Di-ethyl pyro-carbonate.

Introduction

Salmonella, a Gram-negative bacilli member of the family Enterobacteriaceae, ranging approximately 2-3X0.4-0.6µm in size is a causative agent of enteric (typhoid) fever and gastroenteritis. Over 2000 serotypes of *Salmonella* sp infects human and virtually all known wild and domestic animals, including birds, reptiles, and insects. Nowaday's *Salmonella* continued to develop resistance against most antibiotics (Baggesen and Wegener, 1994; Baggesen and Aarestrup, 1998; Molbak *et al.,* 1999).The studies on PhoP/PhoQ regulation revealed it as a best-characterized transcriptional regulation, a two-component system required for *Salmonella* pathogenesis, controls the expression of more than 40 genes (Groisman *et al.,* 1989). The phoP/ phoQ DNA binding Proteins possess positively charged amino acids like Arginine, Lysine and Histidine acts as a DNA Binding site. DNA binding proteins play an important role in biological processes like DNA replication, transcription, and regulation of cell cycles of all organisms. Among the protein families of bacteria, and eukaryotes, the DNA binding protein family are the most studied ones which are small transcriptional factors containing independent domains for the recognition and binding of DNA.

Protein-nucleic acid interactions play many important cellular roles, including regulating DNA replication, controlling gene expression and DNA housekeeping, such as maintaining a super coiled state, performing damage and mismatch repair, allowing recombination, restricting foreign DNA, and the processes of DNA ligation, methylation and degradation (Chia-lung li *et al.,* 2003).A wealth of information regarding how proteins select DNA target sites has been discovered through biochemical, structural and statistical analysis in site-specific DNA-binding proteins, such as transcription factors (Burley, 1994; Jones *et al*., 1999; Wolberger, 1999; Pabo and Nekludova, 2000) .

A transcription factor occurs in monomers and dimmers. Activation of transcription factors occur in dimmer form which requires the formation of large, multi component protein–DNA complexes choosing their route of assembly from many potential pathways. *In vivo*, the pathway chosen will depend on the availability of components and the relative rate

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constants of individual steps along each pathway, as well as on the pro open sites of intermediates to engage in alternative interactions with other nuclear components. A large number of DNA-binding proteins show elevated affinity to damaged DNA but they are probably not involved directly in DNA repair. Among them are the abundant chromatin proteins HMG-1, 2 and histone H1, which show preferential binding to DNA damaged by *cis*-DDP. Poly (ADP-ribose) polymerase (PARP) binds to single or double strand ends, however its involvement in DNA repair still remains unknown. It has been proposed that such proteins may compete with DNA repair proteins, making the repair less efficient.

Defects in DNA repair genes result in several serious diseases that are caused by gradual accumulation of non-repaired DNA lesions. Patients with Xeroderma pigmentosum (XP) suffer from severe UV-sensitivity, which is caused by defects in several genes involved in different steps of NER. Several other human disorders like Cockayne syndrome, ataxia telangiectasia, Nijmegen breakage syndrome are also caused by defects in DNA repair genes. Amino acids involved in DNA-Protein interaction can be studied using Chemical modifiers. The Histidine residues that are present in the active site of many enzymes like neutral endopeptidase 24.1, S1 nuclease, pig kidney 3,4-dihydroxyphenylalanine decarboxylase, which are involved in the interaction of Protein with DNA were modified by many chemicals such as halo carboxylic acids, amides and Diethyl pyro carbonate (DEPC). Among these, Diethyl pyro carbonate (ethoxyformic anhydride), most widely used chemical modifier for Histidine residues. It reacts with Histidine and substitutes one of the two nitrogen atom present in the imidazole ring to give carboethoxyhistidine. It partially modifies Tyrosine and Lysine.

Detection methods for DNA-Protein interaction

Gel Mobility Shift Assay (GMSA) and Spectrofluorimeter were commonly used to detect the DNA-Protein interactions. Gel Mobility Shift Assay provides a simple method to study DNA-Protein interaction. This method is based on the principle that a DNA-Protein complex will have a different mobility during electrophoresis than unbound DNA. It is also called as Gel retardation assay (Fried, 1989).

Materials and Methods

Using Kirby-Bauer principle multiple drug resistant (MDR) *S. typhi* was collected from Erode slaughter house for the present study. Antibiotic susceptibility test of the organism was carried out for eleven antibiotics (Table-1). Plasmid DNA of the MDR was isolated through alkaline lysis mini prep method and PhoP and PhoQ proteins were extracted using nuclear extraction buffer after culturing the isolated MDR *S. typhi*. Extracted PhoP/Q proteins were quantified through Lowry's (1950) method and purified in SDS-PAGE analysis.

Antimicrobial Susceptibility Test

Antimicrobial susceptibility tests were performed by Kirby-Bauer disc diffusion method. Overnight cultures in peptone water were lawned over the Mueller-Hinton agar (Hi-media). The antibiotic discs were used at the following concentration: Ampicillin (A) 30 mcg, Amikacin (Ak) 30 mcg, Amoxicillin (Am) 25 mcg, Chloramphenicol (C) 25 mcg, Ciprofloxacin (Cf) 30 mcg, Co-trimaxazole (Co) 25 mcg, Gentamicin (G) 30 mcg, Nalidixic acid (Na) 30 mcg, Oflaxacin (O) 30 mcg, Rifampicin (R) 30 mcg, Tetracycline (T) 10 mcg (Hi-media).

DNA and PhoP/Q protein interaction

The DNA and PhoP/Q interaction was studied by conducting Gel mobility shift assay (GMSA) for DNA and PhoP/Q mixture and chemical modification of basic amino acids of PhoP/Q protein.

GMSA of DNA and PhoP/Q mixture

Isolated plasmid DNA and PhoP/Q protein extract without incubation and with incubation at 10°C for 30 minutes after mixing plasmid DNA and PhoP/Q proteins in the ratio's of 0.5, 1.0, 1.5, 2.0, 2.5 and 3µl per 10µl of plasmid DNA and simultaneously made up to 20µl with distilled water were run for six hours at10°C in 0.7% agarose gel. The concentration of protein mixed with plasmid DNA was 0.4µg/µl.

GMSA of DNA and modified PhoP/Q

Di-ethyl pyro carbonate (DEPC) mixed with extracted PhoP/Q nuclear protein in the ratios of 0.5µl, 1.0µl and 1.5µl per 10µl and incubated at 10°C for 30 minutes. From each of this incubated mixture 3µl nuclear protein was mixed with 10µl of isolated plasmid DNA and incubated at 10°C for 30 minutes. Incubated plasmid DNA and

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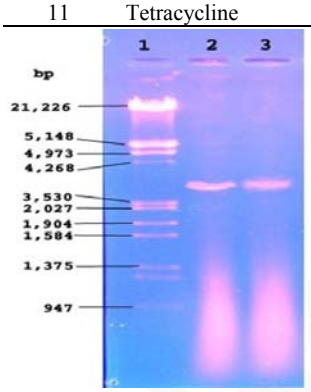
DEPC modified protein extract was subjected to GMSA in 0.7% agarose gel for six hours at 10°C.

Results

The zone of inhibition of MDR *S. typhi* ranged from 9 to 20mm as per Kirby-Bauer disc diffusion method. High sensitivity was noted for Ciproflaxacin, Oflaxacin and Co-trimaxazole with zone of inhibition of 20, 18 and 16mm respectively. Smaller zone of inhibition was recorded with tetracycline followed by Amikacin, Amoxicillin, Ampicillin, Rifampicin, Gentamicin, Nalidixic acid and Chloramphenicol in the increasing order up to 13mm (Table-1).

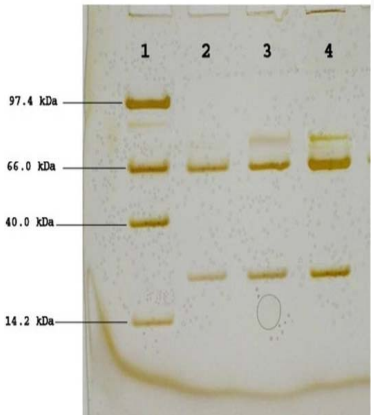
Table 1: Sensitivity pattern of *S.typhi* isolated from slaughter house

S.No	Antibiotic	Concentration (µg)/Disc	Zone of Inhibition (mm)	Results
1	Ampicillin	30	11	R
2	Amikacin	30	10	R
3	Amoxicillin	25	10	R
4	Chloramphenicol	25	13	R
5	Ciproflaxacin	30	20	S
6	Co-trimaxazole	25	16	S
7	Gentamicin	30	11	R
8	Nalidixic acid	30	12	R
9	Oflaxacin	30	18	S
10	Rifampicin	30	11	R
11	Tetracycline	10	9	R



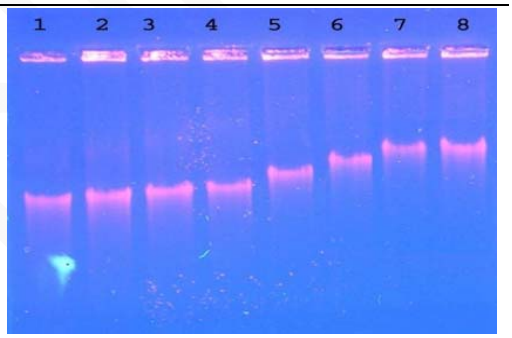
Lane 1: Marker Lane 2 and 3: Plasmid DNA

Fig.1: Plasmid DNA in 1% Agarose Gel.



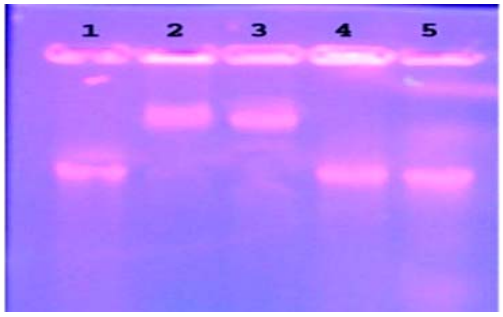
Lane 1: DNA Molecular wt. Lane 2-4: 10,20,,30 µg of DNA binding protein

Fig.2: SDS PAGE of DNA Binding Protein



Lane 1: Free Plasmid DNA; Lane 2-6: DNA complexed with 0.5, 1.0, 1.5, 2.0, 2.5µL of DNA binding protein; Lane 7-8: DNA complexed with 3.0 µL of DNA binding protein.

Fig.3: Gel Mobility shift Assay of DNA-Protein



Lane 1: Free Plasmid DNA; Lane 2: DNA complexed with protein; Lane 3: DNA protein modified with 0.5Mm DEPC; Lane 4: DNA protein modified with 1Mm DEPC; Lane 5: DNA protein modified with 1.5Mm DEPC.

Fig.4: Gel Mobility shift Assay of DNA-Protein treated with DEPC

The plasmid DNA was isolated and purified from MDR *S. typhi* under the purity of ≥ 1.8 ratio of 260/280nm absorbance (Figure.1). Further the Nuclear protein was separated through nuclear extraction buffer and PhoQ proteins were distinctly identified through SDS PAGE (Figure.2 Gel mobility shift assay (GMSA) of 0.9µg/ml of plasmid DNA added with 3µg/ml of PhoP/Q protein exhibited slowest shift when compared to the other concentrations of free plasmid DNA shift (Figure.3).

Diethyl pyrocarbonate (DEPC) treated PhoP and PhoQ proteins showed better shift from 1mM and 1.5mM similar to free DNA where as at 0.5mM concentration of DEPC there was less shift with 3µg/ml of modified protein with 0.9µg/ml of free plasmid DNA (Figure.4).

Discussion

Enteric fever caused by *S. typhi* has been uncontrolled problem in developing world due to its high virulence. It has been reported that *S. typhi* is resistant to two or more antibiotic among the twelve commonly used antibiotics of the day, importantly the Chloramphenicol, Ampicillin and Cotrimaxole. In the present study showed that the isolated strain was susceptible for only three antibiotics Ciproflaxacin, Co-trimaxazole and Oflaxacin out of eleven commonly used antibiotics which includes the Chloramphenicol and Ampicillin were tabulated in Table-1. However, it could detected that continuous use of antibiotics for treatment further increases its spectrum of drug resistance, which is evident through that in the present study the isolates are resistant to Tetracycline and Ciprofloxacin which were once susceptible . Therefore there is an urgent need of novel therapy which could face this mutant strains at genomic level. Hence, the present study was initiated to repress the Salmonella virulence by novel gene therapeutic approach.

Virulence is controlled at transcriptional level by several proteins like RpoS sigma factor (Fang *et al*., 1992), the cyclic AMP-binding protein and the two components of regulatory systems OmpR-EnvZ (Dorman *et al*., 1989) and PhoP/PhpQ (Galan and Curtiss, 1989). PhoQ was predicted as phosphorylating and dephosphorylating agent in response to environmental changes were mutation in PhoP/PhoQ as well as constitutive allele mapping to PhoQ result in attenuation for virulence (Fields, *et al*., 1986, Miller and Mekalanos, 1990). Positively charged amino acids help in the interaction of phoP/ phoQ DNA binding Proteins with DNA. To study these interaction Gel shift mobility assay was commonly employed. Hence the plasmid DNA was isolated from MDR *S. typhi* with the purity of ≥ 1.8 ratio of 260/280nm absorbance and was found to be around 2,700 bp as per 100 bp DNA ladder of 5M miniprep kit (Figure.1). Further Nuclear protein obtained through nuclear extraction buffer and quantified 27µg/ml through Lowry *et al*., (1958). 20 kDa PhoP and 66 kDa PhoQ proteins were distinctly identified through SDS PAGE and represented in Figure.2.The Gel mobility shift assay of phoP/ phoQ DNA binding Proteins bound to DNA moves slower than the free DNA inside the agarose gel. 3µg/ml of phoP/ phoQ DNA binding Proteins was found to be sufficient to interact with 10µl of plasmid DNA was shown in Figure 3. The phoP/phoQ DNA binding Proteins starts inhibiting by DEPC at a minimum concentration of 1mM. At 0.5 mM there was no inhibition, hence the phoP/ phoQ DNA binding Proteins binds with the DNA and results in a Mobility Shift. 1, 1.5, 2 and 2.5mM concentration of DEPC modifies the histidines present in the phoP/ phoQ DNA binding proteins is revealed by Figure 4; hence no shift in the mobility of DNA was identified.

The results of Gel mobility shift assay confirmed that DEPC modifies the histidines present in the binding sites of phoP/ phoQ DNA binding Proteins. Histidine modified phoP/ phoQ DNA binding Proteins fails to interact with DNA which may result in unexpression of virulence genes. Thus this mechanism at a molecular level might be paving a path in the treatment of enteric fever without leading to the further MDR Salmonella strains.

References

Baggesen, D.L. and Aarestrup, F.M. 1998. Characterization of recently emerged multiple antibiotic-resistant Salmonella enterica serovar Typhimurium DT104 and other multiresistant phage types from Danish pig herds. *The Veterinary Record*, 143: 95–97.

Baggesen, D.L.and Wegener, H.C. 1994. Phage types of *Salmonella enterica* ssp. enterica serovar *typhimurium* isolated from production animals and humans in Denmark. *Acta Veterinaria Scandinavica,* 35: 349–354.

Burley, S.K. 1994. DNA-binding motifs from eukaryotic transcription factors. *Curr Opin Struct Biol,* 4: 3–11.

Chia-Lung Li, Lien-I Hor, Zee-Fen Chang, Li-Chu Tsai Wei-Zen Yang, and Hanna S. Yuan. 2003. DNA binding and cleavage by the periplasmic nuclease Vvn: a novel structure with a known active site. *EMBO J, 22*:4014-4025.

Dominici, P., Tancini, B. and Voltatottorni, B .C.1985 .Chemical modification of pig kidney 3, 4-dihydroxyphenylalanine decarboxylase with diethyl pyrocarbonate. *J. Biol Chem.,* 260: 10583-10589.

Dorman, C. J., Chatfield, S., Higgins, C.F., Hayward, C. and Dougan, G.1989. Characterization of porin and ompR mutants of a virulent strain of *Salmonella typhimurium*: ompR mutants are attenuated in vivo. *Infect. Immun*. 57:2136–2140.

Fang, F. C., Libby, S.J., Buchmeier, N.A., Loewen, P.C., Switala, J, J., Harwood, J. and Guiney, D.G. 1992. The alternative sigma factor katF (rpoS) regulates *Salmonella* virulence. *Proc. Natl. Acad. Sci. USA,* 89:11978–11982.

Fields, P. I., Swanson, R.V., Haidaris, C.G. and Heffron, F. 1986. Mutants of *Salmonella typhimurium* that cannot survive within the macrophage are a virulent. *Proc. Natl. Acad. Sci. USA,* 83:5189–5193.

Fried, M.1989. Measurement of protein-DNA interaction parameter by Electrophoretic Gel mobility shift. *Electrophoresis*, 10:366-376.

Galan, J. E. and Curtiss, R. III. 1989. Virulence and vaccine potential of phoP mutants of *Salmonella typhimurium*. *Microb. Pathog, 6*:433–443.

Galina Abdulova, L. and Evgueni, D. Ananiev, Petar Grozdanov, 2002. Isolation and Garci´a Ve´scovi, E., Soncini, F.C. and Groisman, E.A. 1994. The role of the PhoP/PhoQ regulon in Salmonella virulence. *Res. Microbiol*., 145:473–480.

Groisman, E. A., Chiao, E., Lipps, C.J. and Heffron, F. 1989. *Salmonella typhimurium* phoP virulence gene is a transcriptional regulator. *Proc. Natl. Acad. Sci. USA*, 86:7077–7081.

Jones, S., Heyningen, P.V., Berman, H.M. and Thornton, J.M. 1999. Protein–DNA interactions: a structural analysis. *J. Mol. Biol.,* 287: 877-896.

Karim Roder and Michael Schweitzer,2001. Running-buffer composition influences DNA-protein and protein–protein complexes detected by electrophoretic mobility- shift assay (EMSA). *Biotechnol. Appl. Biochem*., 33:209–214.

Miller, S.I., and Mekalanos, J.J. 1990. Constitutive expression of the phoP regulon attenuates *Salmonella* virulence and survival within macrophages. *J. Bacteriol*., 172:2485–2490.

Molbak, K., Baggesen, D.L., Aarestrup, F.M., Ebbesen, J.M., Engberg, J., Frydendahl, K., Gerner-Smidt, P., Petersen, A.M. and Wegener, H.C. 1999. An outbreak of multidrug-resistant, quinolone-resistant Salmonella enterica serotype Typhimurium DT104. *The New England Journal of Medicine,* 341:1420–1425.

Olano, J., Soler, J., Busto, F. and De Arriaga, D.1999 .Chemical modification of NADP-isocitrate dehydrogenase from *Cephalosporium acremonium* evidence of essential histidine and lysine groups at the active site. *Eur. J. Biochemistry*, 261: 640-649.

Pabo, C.O. and Nekludova, L. 2000. Geometric analysis and comparison of protein–DNA interfaces: why is there no simple code for recognition. *J. Mol. Biol.*, 301: 597–624.

Purification of nuclear DNA from excised cotyledons of cucurbita. *Bulg. J. Plant Physiol.* 28(3–4):1–3.

Isobel A.Wadman, Hirotaka Osada, Gerald G.Gru¨ tz, Alan D.Agulnick, Heiner Westphal Alan Forster and Terence H.Rabbitts.1997. The LIM-only protein Lmo2 is a bridging molecule assembling an erythroid, DNA-binding complex which includes the TAL1, E47, GATA-1 and Ldb1/NLI proteins. *EMBO J* 16(11):3145–3157

Wolberger, C. 1999. Multiprotein –DNA complexes in transcription regulation. *Annu Rev Biophys Biomol Struct*., 28:29–56.

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