DRUG DISCOVERY

16(37), 2022

Characteristic Study of sul3 gene expression, protein profiling and multi resistance in *Salmonella typhi*

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To Cite:
Sudhin AS. Characteristic Study of sul3 gene expression, protein profiling and multi resistance in *Salmonella typhi*. *Drug Discovery*, 2022, 16(37), 1-8

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Peer-Review History
Received: 12 October 2021
Reviewed & Revised: 18/October/2021 to 31/December/2021
Accepted: 3 January 2022
Published: 6 January 2022

Peer-review
External peer-review was done through double-blind method.

ABSTRACT

Salmonella can be found worldwide, although the distribution of serovars may vary. *Salmonella* are well known pathogens, highly adaptive and potentially pathogenic for humans and animals. *Salmonella* infections are capable of producing serious infections that are often food borne and present as gastroenteritis. Serotypes adapted to men, such as *Salmonella typhi* and *Salmonella paratyphi*, usually cause severe diseases in humans as a septicaemic typhoid syndrome (enteric fever). The present study investigates the occurrence of sul3 gene in the DNA, characterization of protein to check the virulence and analyze the multi resistance of *Salmonella typhi* to various drugs. The present study confirmed the expression of sul3 gene which showed the resistance to Sulfonamides. The growth in the medium containing drugs showed multiresistance of *S.typhi.*

Keywords: Salmonella; Multiresistance; Typhoid; Antibiotic; Gene expression; Sul3 gene.

1. INTRODUCTION

Salmonella typhi is a facultative anaerobic gram negative rod shaped bacteria belonging to the family *Entero bacteriaceae*. Most of the members of this genus are motile by peritrichous flagella except salmonella entrica serovar pullorum and salmonella entrica gallinarum and non-motile strains from dysfunctional flagella (Sood et al., 2002). Salmonella catabolises D-glucose and other carbohydrates are responsible for production of acid and gas. Salmonella are oxidase negative and catalyse positive, grow on citrate as a sole carbon source, generally produce hydrogen sulfide, decarboxylate lysine and ornithine and donot hydrolyze urea. Many of these traits have formed the basis for the presumptive biochemical identification of salmonella isolates. Bacterial antimicrobial resistance has become a worldwide public health problem with direct impact on food safety, being crucial the monitoring of food-borne pathogens that possess important animal reservoirs, such as *Salmonella*, as proposed by the EU legislation (Perreten and Boerlin, 2003).

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Salmonellosis is one of the most frequent food-borne diseases in almost all industrialized countries. The widespread use of antimicrobial agents in food-animal production has contributed to the occurrence of *Salmonella* with decreased susceptibility to antibiotics, which can be transmitted to humans through food products, particularly those of animal origin (Cruchaga et al., 2001; Perreten and Boerlin, 2003). The increasing number of infections with antimicrobial drug-resistant *Salmonella*, including the emergence of multidrug-resistant (MDR) *Salmonella enterica* serotype Typhimurium phage type DT104, extended-spectrum β-lactamase (ESBL)-producing *Salmonella* and fluoroquinolone-resistant *Salmonella* strains, deserves special attention (Guillemont, 1999). The formation of genetic elements that co integrate antibiotic resistance and virulence determinants, may compromise the therapeutic options in cases of invasive *Salmonella* infections (Helms et al., 2002). The present study investigates the occurrence of sul3 gene in the DNA, characterization of protein to check the virulence and analyze the multi resistance of *Salmonella typhi* to various drugs.

2. MATERIALS AND METHODS

2.1. Sample collection

The selected microbial culture [Salmonella typhi] was purchased from a clinical laboratory, Chennai.

2.2. Sub-culturing and cultivation

The selected microorganism salmonella typhi was sub-cultured in Luria Bertani broth and incubated at 37°C for 24 hours.

2.3. Gene Expression Studies

2.3.1. Isolation and quantification of DNA

2 ml of culture was transferred into a centrifuge tube and was centrifuged at 8000 rpm for 4 min. The pellet was collected and lysis solution was added and incubated at 40° C for 45 min. After the incubation SDS was added and heated the sample for 10 min. then it was cooled to room temperature and equal volume of phenol: chloroform: isoamylalcohol in the ratio 25:24:1 was added and mixed well. Then the sample was centrifuged at 8000 rpm for 4 min and the aqueous phase was collected. To the aqueous phase 95% ethanol was added and mixed well. Again it was centrifuged at 5000rpm for 4 min and pellet was collected. The pellet was washed with ethanol and dissolved in TE buffer. The amount of DNA was quantified using NANOVUE. The quantified sample was run in 0.9% agarose gel and visualized under UV transilluminator.

2.3.2. Purification by elution

The gel portion containing DNA was cut with a sterile blade and transferred to a centrifuge tube. The sample was heated at 70°C for 3 min. 1 volume of phenol equilibrated with 1M tris was added and vortexed for 1 min. Then the sample was centrifuged and the aqueous phase was collected. To that 1 volume of chloroform: isoamylalcohol was added and mixed. Then n-butanol was added and centrifuged. Upper phase was discarded and 0.1 volume of sodium acetate and 2.5 volume of ice cold ethanol were added. DNA was precipitated at -20°C for overnight or -70°C for 1 hour. The precipitate was collected by centrifugation and washed with 80% ethanol and dried in vacuum. The dried pellet was suspended in TE buffer. The purified DNA was quantified using nanovue.

2.3.3. Primer designing and PCR

PCR is the technique which has been used for the amplification of complementary DNA formed from the mRNA. The sul3 gene sequence and accession number was obtained from the NCBI. The accession number of the sul3 gene is AY316203. The primer was designed using the tool primer 3. Sample DNA along with left primer, right primer, PCR master mix and double distilled water was taken in a PCR tube and mixed well. The sample was loaded in the reactor and set the program.

2.3.4. PCR program

Initial denaturation temp: 94°C for 3 min
Denaturation temp : 94°C for 1 min
Annealing temp : 58°C for 1 min
Extension temp : 72°C for 1 min
Final extension temp : 72°C for 5 min
Cycles : 35

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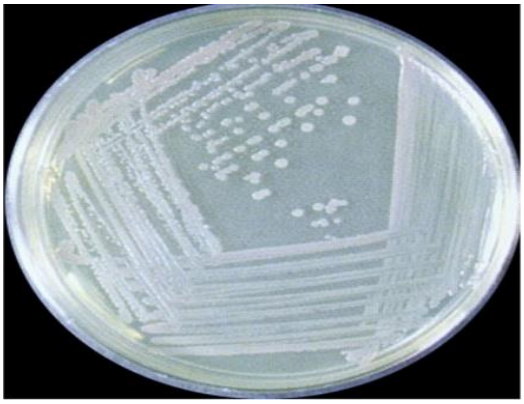

Figure 1
Salmonella typhi in culture media

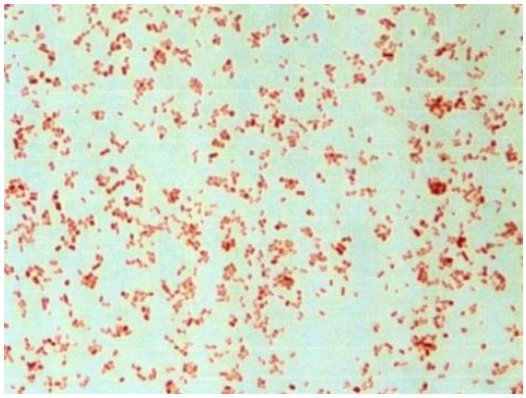

Figure 2
Gram positive rod shaped bacteria

Figure 3
Nanovue for quantification of DNA

Figure 4
Absorbance and concentration of DNA

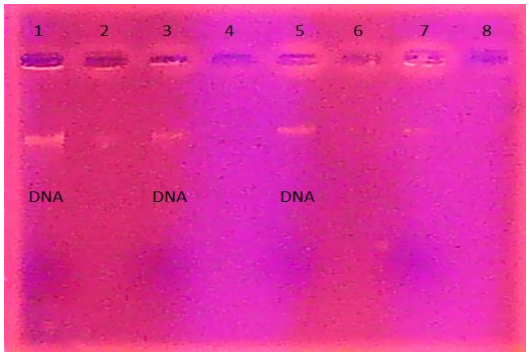

Figure 5
Lane 1, 3, and 5 contains the DNA isolated from Salmonella typhi

2.4. Protein profiling

2.4.1. Protein extraction

2 ml of salmonella culture was taken in a centrifuge tube and centrifuged at 8000 rpm for 7 min and the pellet was collected. To the pellet 1 ml of protein extraction buffer was added and mixed well. Then the sample was kept in aqublock at 100°C for 5 min. After that cooled to room temperature and centrifuged at 2000rpm for 5 min. Supernatant was collected and used for estimation.

2.4.2. Protein estimation

The amount of proteins present in the sample was estimated by using Lowry's method.

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2.4.3. Protein purification by column chromatography

Silica gel was suspended in large volume of elution buffer until the gel was fully swollen and kept in water bath for 2-4 hours. The slurry was poured into the column and waited until the gel settled down to desired height and the column was equilibrated thoroughly by passing through the column buffer. The sample was added in the column buffer on to the top of the bed. The protein molecules passed through the gel space based on their molecular weight.

Primer3 Output

```
PRIMER PICKING RESULTS FOR gi|32450905|gb|AY316203.1| Salmonella enterica subsp.
enterica serovar Typhimurium dihydropteroate synthase (sul3) gene, complete cds

No mispriming library specified
Using 1-based sequence positions
OLIGO            start  len      tm     gc%   any   3' seq
LEFT PRIMER        547   20   60.05   45.00  6.00  0.00 GTTGAAACGAATCCGGAAGA
RIGHT PRIMER       770   20   60.12   45.00  5.00  3.00 TTCCGTGACACTGCAATCAT
SEQUENCE SIZE: 930
INCLUDED REGION SIZE: 930

PRODUCT SIZE: 224, PAIR ANY COMPL: 5.00, PAIR 3' COMPL: 2.00
```

Figure 6

Primers for the expression of sul3 gene

2.4.4. Thin layer chromatography

The glass slab was wiped with ethanol and incubated for 1 hour in 100°C for the activation. 20 gram of silica gel was made into slurry with distilled water and a thin layer was spread over the activated glass slab with the help of a glass rod. It was then air dried for 10 minutes and kept inside the hot air oven for 1 hour for complete drying. The extracted protein was loaded in the thin layer of silica gel and immersed in the solvent system (Butan-2-ol: Water: Acetic acid at ratio of 80: 20: 20) for the separation of protein. After separation slide was dried and treated with Ninhydrin. Purple colour was formed. This shows the confirmation of presence of proteins.

2.4.5. SDS PAGE & Molecular weight determination

The purified protein was subjected to SDS page along with the marker to determine the molecular weight. The SDS setup was taken. Separating gel and stacking gel was prepared and casted in between the glass plates and allowed to solidify. After solidification the gel plate was fixed in the apparatus and the electrode buffer was added in the upper and lower tank. Then the samples with sample buffer were added in the wells and 50 V current was applied. The gel was viewed under the UV trans illuminator and molecular weight was determined by calculating the RF value.

$$R_f = \frac{\text{Distance moved by the sample}}{\text{Distance moved by the dye}}$$

2.5. Multi resistance analysis

2.5.1. Plasmid DNA isolation

Plasmid bearing culture was transferred into a centrifuge tube and was centrifuged at 4000 rpm for 5 min. pellet was collected and lysis solution was added and mixed well. It was then incubated at 37°C for 30 minutes. After incubation phenol chloroform isoamylalcohol was added and centrifuged at 12000 rpm for 10 min. The upper aqueous phase was collected carefully by avoiding the white interphase. To that 5µl of blue II solution was added and the sample was loaded in 0.9% agarose gel and electrophoresis was performed.

2.5.2. Multi resistant studies

The plasmid DNA was isolated from the salmonella typhi. The Luria Bertani agar and Muller Hinton agar plates were prepared along with anti-typhoid drugs like cefixime and antibiotics like chloramphenicol, amphicillin, and amoxicillin. Then the culture was incubated overnight for checking the multi resistance.

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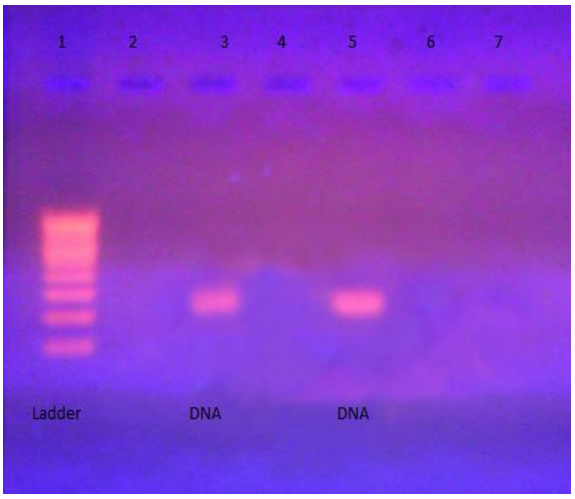

Figure 7
PCR amplified DNA

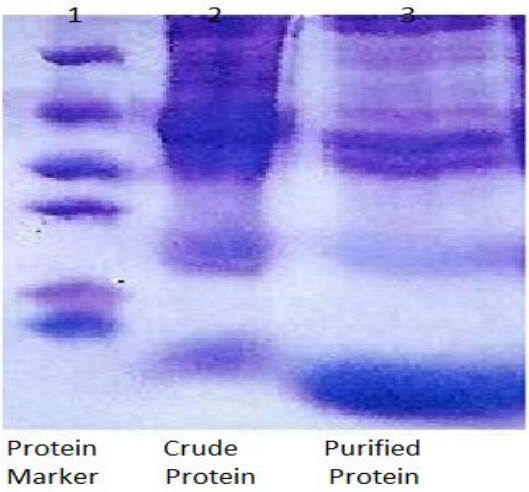

Figure 10
Separation of Protein by SDS-PAGE

Figure 8
Purification of crude protein extract by column chromatography

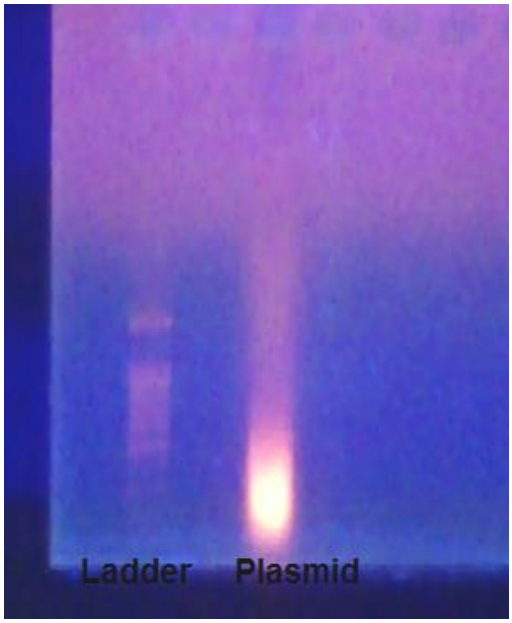

Figure 11
Plasmid from Salmonella typhi

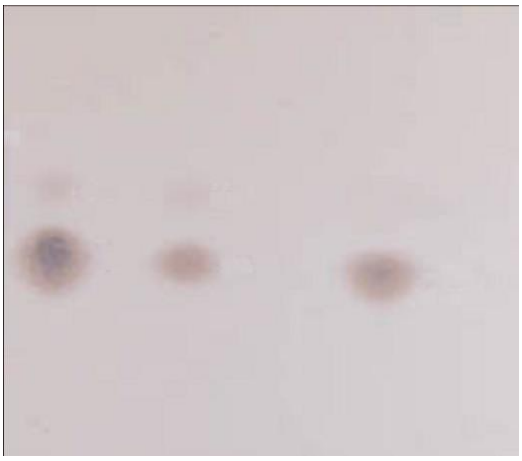

Figure 9
Confirmation of protein by Thin Layer Chromatography

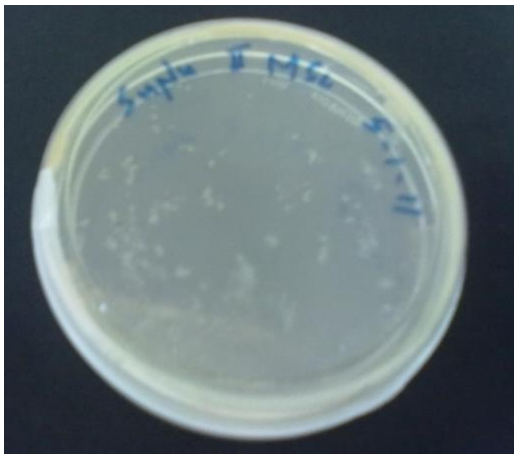

Figure 12
Ampicillin resistance

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3. RESULTS AND DISCUSSION

3.1. Molecular Characterization

Salmonella is a genus of rod shaped, gram negative, non-spore forming, and predominantly motile enterobacteria. The morphology of the bacteria was shown in Fig.1 and 2. The DNA was isolated from salmonella typhi using alkaline lysis method and quantified using nanovue. The purity of the isolated sample was identified as 1.73 and the concentration was 1527 ng/µl shown in Fig.3 and 4. The isolated DNA was subjected to electrophoresis and the gel was viewed under uv-trans illuminator. Bands were observed (Fig.5) and it confirms the presence of DNA. The DNA was purified by elution and the primer was designed using the primer tool for the amplification (Fig.6). The amplification was done by PCR. The amplified product was subjected to electrophoresis along with markers. The bands were obtained and it showed the expression of *sul3* gene (Fig.7).

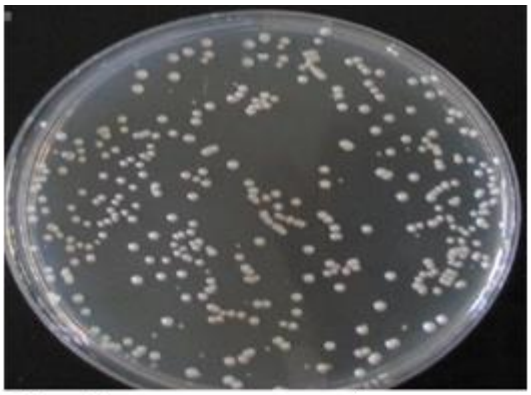

Figure 13
Chloramphenicol resistance

Figure 14
Amoxicillin resistance

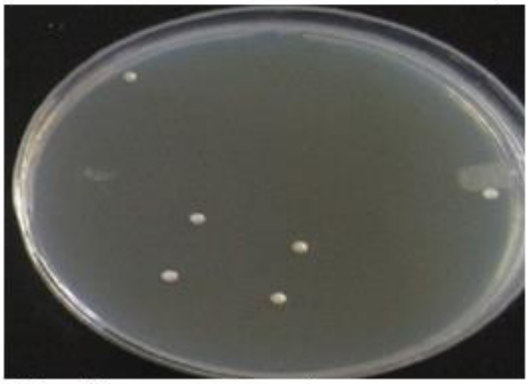

Figure 15
Typhoid drug [cefixime]

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3.2. Protein profiling

The protein was extracted from Salmonella typhi and was estimated by Lowry's method. The crude protein was purified by column chromatography method (Fig.8) and confirmed by thin layer chromatography shown in Fig.9. The purified protein was subjected to SDS PAGE along with marker to determine the molecular weight of the sample (Fig. 10).

3.3. Multiresistance analysis

The plasmid DNA was isolated from salmonella typhi shown in Fig.11 and the antibiotic resistance of salmonella typhi was confirmed by culturing it in medium containing various antibiotics. The growth was observed after the incubation period and it showed the resistance of various drugs Fig.12-15.

4. CONCLUSION

The present study reveals the expression of sul3 gene from the human clinical isolates of *Salmonella typhi*. Molecular characterization was done by isolating the DNA and the primer was designed for the expression of the resistant gene sul3. The band formation in the agarose gel confirms the presence of sul3 gene. Protein profile was studied by extracting the crude protein. The extracted protein was estimated by Lowry's method and the protein was purified by column and Thin Layer Chromatography. The molecular weight was determined by SDS-PAGE. Multi resistance of the organism was checked by isolating the plasmid from the culture medium with ampicillin. The organism was also checked for the growth in the medium containing various drugs. The growth in the plates showed the multi resistance of the organism.

SUMMARY OF RESEARCH

1. Morphological studies of the collected samples were identified by staining and biochemical test method.
2. Molecular characterization of the sample was studied by isolating the DNA.
3. Isolated DNA were purified by elution method and used for amplification.
4. To express the sul3 gene, primer were designed and performed in thermal cycler for the amplification of specific gene.
5. To study the protein profile, protein were extracted from the sample and estimated by Lowry's method.
6. The extracted crude protein was purified by column chromatography and confirmed the presence of protein by thin layer chromatography.
7. The molecular weight of the extracted protein was determined by running the sample in SDS-PAGE along with marker.
8. Multiresistance of the Salmonella typhi was determined by isolating the plasmid and cultivates the organism with various drugs.

FUTURE ISSUSES

1. Expression of sul3 gene can be confirmed by sequencing
2. Characterization of the protein has to be done by HPLC to check the proteins which cause virulence.
3. Highly sensitive Drug has to be designed to treat the disease caused by *Salmonella* to reduce the virulence and side effects.

Acknowledgments

We are grateful to our management, Hindustan College of Arts & Science for providing opportunity to perform this research work. We extend our thanks to friends and family members for their kind and constant support to complete this research.

Funding:

This study has not received any external funding.

Ethical approval

The ethical guidelines are followed in the study for microbial experimentation.

Conflict of Interest:

The authors declare that there are no conflicts of interests.

Data and materials availability:

All data associated with this study are present in the paper.

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